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(54) **Combinations of hepatitis C virus (HCV) antigens for use in immunoassays for anti-HCV antibodies.**

(57) **Combinations of HCV antigens that have a broader range of immunological reactivity than any single HCV antigen. The combinations consist of an antigen from the C domain of the HCV polyprotein, and at least one additional HCV antigen from either the NS3 domain, the NS4 domain, the S domain, or the NS5 domain, and are in the form of a fusion protein, a simple physical mixture, or the individual antigens commonly bound to a solid matrix.**

EP 0 450 931 A1

Technical Field

The present invention is in the field of immunoassays for HCV (previously called Non-A, Non-B hepatitis virus). More particularly, it concerns combinations of HCV antigens that permit broad range immunoassays for anti-HCV antibodies.

Background

The disease known previously as Non-A, Non-B hepatitis (NANBH) was considered to be a transmissible disease or family of diseases that were believed to be viral-induced, and that were distinguishable from other forms of viral-associated liver diseases, including that caused by the known hepatitis viruses, i.e., hepatitis A virus (HAV), hepatitis B virus (HBV), and delta hepatitis virus (HDV), as well as the hepatitis induced by cytomegalovirus (CMV) or Epstein-Barr virus (EBV). NANBH was first identified in transfused individuals. Transmission from man to chimpanzee and serial passage in chimpanzees provided evidence that NANBH was due to a transmissible infectious agent or agents. Epidemiologic evidence suggested that there may be three types of NANBH: a water-borne epidemic type; a blood-borne or parenterally transmitted type; and a sporadically occurring (community acquired) type. However, until recently, no transmissible agent responsible for NANBH had been identified, and clinical diagnosis and identification of NANBH had been accomplished primarily by exclusion of other viral markers. Among the methods used to detect putative NANBH antigens and antibodies were agar-gel diffusion, counterimmunoelectrophoresis, immunofluorescence microscopy, immune electron microscopy, radioimmunoassay, and enzyme-linked immunosorbent assay. However, none of these assays proved to be sufficiently sensitive, specific, and reproducible to be used as a diagnostic test for NANBH.

In 1987, scientists at Chiron Corporation (the owner of the present application) identified the first nucleic acid definitively linked to blood-borne NANBH. See, e.g., EPO Pub. No. 318,216; Houghton et al., Science 244:359 (1989). These publications describe the cloning of an isolate from a new viral class, hepatitis C virus (HCV), the prototype isolate described therein being named "HCV1." HCV is a Flavi-like virus, with an RNA genome.

U.S. Patent Application Serial No. 456,637 (Houghton et al.), incorporated herein by reference, describes the preparation of various recombinant HCV polypeptides by expressing HCV cDNA and the screening of those polypeptides for immunological reactivity with sera from HCV patients. That limited screening showed that at least five of the polypeptides tested were very immunogenic; specifically, those identified as 5-1-1, C100, C33c, CA279a, and CA290a. Of these five polypeptides, 5-1-1 is located in the putative NS4 domain; C100 spans the putative NS3 and NS4 domains; C33c is located within the putative NS3 domain and CA279a and CA290a are located within the putative C domain. The screening also showed that no single polypeptide tested was immunologically reactive with all sera. Thus, improved tests, which react with all or more samples from HCV positive individuals, are desirable.

Disclosure of the Invention

Applicants have carried out additional serological studies on HCV antigens that confirm that no single HCV polypeptide identified to date is immunologically reactive with all sera. This lack of a single polypeptide that is universally reactive with all sera from individuals with HCV may be due, *inter alia*, to strain-to-strain variation in HCV epitopes, variability in the humoral response from individual-to-individual and/or variation in serology with the state of the disease.

These additional studies have also enabled applicants to identify combinations of HCV antigens that provide more efficient detection of HCV antibodies than any single HCV polypeptide.

Accordingly, one aspect of this invention is a combination of HCV antigens comprising:

- (a) a first HCV antigen from the C domain; and
- (b) at least one additional HCV antigen selected from the group consisting of
 - (i) an HCV antigen from the NS3 domain;
 - (ii) an HCV antigen from the NS4 domain;
 - (iii) an HCV antigen from the S domain; and
 - (iv) an HCV antigen from the NS5 domain.

In one embodiment, the combination of HCV antigens is in the form of a fusion protein comprised of the antigens. In an alternative embodiment, the combination of antigens is in the form of the individual antigens bound to a common solid matrix. In still another embodiment, the combination of antigens is in the form of a mixture of the individual antigens.

Another aspect of the invention is a method for detecting antibodies to HCV in a mammalian body compo-

ment suspected of containing said antibodies comprising contacting said body component with the above-described combination of HCV antigens under conditions that permit antibody-antigen reaction and detecting the presence of immune complexes of said antibodies and said antigens.

Another aspect of the invention is a method for detecting antibodies to HCV in a mammalian body component suspected of containing said antibodies comprising contacting said body component with a panel of HCV antigens, simultaneously or sequentially, comprising

- (a) a first HCV antigen from the C domain; and
- (b) at least one additional HCV antigen selected from the group consisting of
 - (i) an HCV antigen from the NS3 domain;
 - (ii) an HCV antigen from the NS4 domain;
 - (iii) an HCV antigen from the S domain; and
 - (iv) an HCV antigen from the NS5 domain under conditions that permit antibody-antigen reaction and detecting the presence of immune complexes of said antibodies and said antigens.

Another aspect of the invention is a kit for carrying out an assay for detecting antibodies to HCV in a mammalian body component suspected of containing said antibodies comprising in packaged combination

- (a) said combination of HCV antigens;
- (b) standard control reagents; and
- (c) instructions for carrying out the assay.

Brief Description of the Drawings

In the drawings:

Figure 1 is the nucleotide sequence of the cDNA sense and anti-sense strand for the HCV polyprotein and the amino acid sequence encoded by the sense strand.

Figure 2 is a schematic of the amino acid sequence of Figure 1 showing the putative domains of the HCV polypeptide.

Modes for Carrying Out the Invention

Definitions

"HCV antigen" intends a polypeptide of at least about 5 amino acids, more usually at least about 8 to 10 amino acids that defines an epitope found in an isolate of HCV. Preferably, the epitope is unique to HCV. When an antigen is designated by an alphanumeric code, the epitope is from the HCV domain specified by the alphanumeric.

"Synthetic" as used to characterize an HCV antigen intends that the HCV antigen has either been isolated from native sources or man-made such as by chemical or recombinant synthesis.

"Domains" intends those segments of the HCV polyprotein shown in Figure 2 which generally correspond to the putative structural and nonstructural proteins of HCV. Domain designations generally follow the convention used to name Flaviviral proteins. The locations of the domains shown in Figure 2 are only approximate. The designations "NS" denotes "nonstructural" domains, while "S" denotes the envelope domain, and "C" denotes the nucleocapsid or core domain.

"Fusion polypeptide" intends a polypeptide in which the HCV antigen(s) are part of a single continuous chain of amino acids, which chain does not occur in nature. The HCV antigens may be connected directly to each other by peptide bonds or be separated by intervening amino acid sequences. The fusion polypeptides may also contain amino acid sequences exogenous to HCV.

"Common solid matrix" intends a solid body to which the individual HCV antigens or the fusion polypeptide comprised of HCV antigens are bound covalently or by noncovalent means such as hydrophobic adsorption.

"Mammalian body component" intends a fluid or tissue of a mammalian individual (e.g., a human) that commonly contains antibodies produced by the individual. Such components are known in the art and include, without limitation, blood, plasma, serum, spinal fluid, lymph fluid, secretions of the respiratory, intestinal or genitourinary tracts, tears, saliva, milk, white blood cells, and myelomas.

"Immunologically reactive" means that the antigen in question will react specifically with anti-HCV antibody commonly present in a significant proportion of sera from individuals infected with HCV.

"Immune complex" intends the combination or aggregate formed when an antibody binds to an epitope on an antigen.

Combinations of HCV Antigens

Figure 2 shows the putative domains of the HCV polyprotein. The domains from which the antigens used in the combinations derive are: C, S (or E), NS3, NS4, and NS5. The C domain is believed to define the nucleocapsid protein of HCV. It extends from the N-terminal of the polyprotein to approximately amino acid 120 of Figure 1. The S domain is believed to define the virion envelope protein, and possibly the matrix (M) protein, and is believed to extend from approximately amino acid 120 to amino acid 400 of Figure 1. The NS3 domain extends from approximately amino acid 1050 to amino acid 1640 and is believed to constitute the viral protease. The NS4 domain extends from the terminus of NS3 to approximately amino acid 2000. The function of the NS4 protein is not known at this time. Finally, the NS5 domain extends from about amino acid 2000 to the end of the polyprotein and is believed to define the viral polymerase.

The sequence shown in Figure 1 is the sequence of the HCV1 isolate. It is expected that the sequences of other strains of the blood-borne HCV may differ from the sequence of Figure 1, particularly in the envelope (S) and nucleocapsid (C) domains. The use of HCV antigens having such differing sequences is intended to be within the scope of the present invention, provided, however, that the variation does not significantly degrade the immunological reactivity of the antigen to sera from persons infected with HCV.

In general, the HCV antigens will comprise entire or truncated domains, the domain fragments being readily screened for antigenicity by those skilled in the art. The individual HCV antigens used in the combination will preferably comprise the immunodominant portion (i.e., the portion primarily responsible for the immunological reactivity of the polypeptide) of the stated domain. In the case of the C domain it is preferred that the C domain antigen comprise a majority of the entire sequence of the domain. The antigen designated C22 (see Example 4, *infra*), is particularly preferred. The S domain antigen preferably includes the hydrophobic subdomain at the N-terminal end of the domain. This hydrophobic subdomain extends from approximately amino acid 199 to amino acid 328 of Figure 1. The HCV antigen designated S2 (see Example 3, *infra*), is particularly preferred. Sequence downstream of the hydrophobic subdomain may be included in the S domain antigen if desired.

A preferred NS3 domain antigen is the antigen designated C33c. That antigen includes amino acids 1192 to 1457 of Figure 1. A preferred NS4 antigen is C100 which comprises amino acids 1569 to 1931 of Figure 1. A preferred NS5 antigen comprises amino acids 2054 to 2464 of Figure 1.

The HCV antigen may be in the form of a polypeptide composed entirely of HCV amino acid sequence or it may contain sequence exogenous to HCV (i.e., it may be in the form of a fusion protein that includes exogenous sequence). In the case of recombinantly produced HCV antigen, producing the antigen as a fusion protein such as with SOD, alpha-factor or ubiquitin (see commonly owned U.S. Pat. No. 4,751,180, U.S. Pat. No. 4,870,008 and U.S. Pat. Application Serial. No. 390,599, filed 7 August 1989, the disclosures of which are incorporated herein, which describe expression of SOD, alpha-factor and ubiquitin fusion proteins) may increase the level of expression and/or increase the water solubility of the antigen. Fusion proteins such as the alpha-factor and ubiquitin fusion are processed by the expression host to remove the heterologous sequence. Alpha-factor is a secretion system, however, while ubiquitin fusions remain in the cytoplasm.

Further, the combination of antigens may be produced as a fusion protein. For instance, a continuous fragment of DNA encoding C22 and C33c may be constructed, cloned into an expression vector and used to express a fusion protein of C22 and C33c. In a similar manner fusion proteins of C22 and C100; C22 and S2; C22 and an NS5 antigen; C22, C33c, and S2; C22, C100 and S2, and C22, C33c, C100, and S2 may be made. Alternative fragments from the exemplified domain may also be used.

Preparation of HCV Antigens

The HCV antigens of the invention are preferably produced recombinantly or by known solid phase chemical synthesis. They may, however, also be isolated from dissociated HCV or HCV particles using affinity chromatography techniques employing antibodies to the antigens.

When produced by recombinant techniques, standard procedures for constructing DNA encoding the antigen, cloning that DNA into expression vectors, transforming host cells such as bacteria, yeast, insect, or mammalian cells, and expressing such DNA to produce the antigen may be employed. As indicated previously, it may be desirable to express the antigen as a fusion protein to enhance expression, facilitate purification, or enhance solubility. Examples of specific procedures for producing representative HCV antigens are described in the Examples, *infra*, and in parent application Serial No. 456,637.

Formulation of Antigens for Use in Immunoassay

The HCV antigens may be combined by producing them in the form of a fusion protein composed of two

or more of the antigens, by immobilizing them individually on a common solid matrix, or by physically mixing them. Fusion proteins of the antigen may also be immobilized on (bound to) a solid matrix. Methods and means for covalently or noncovalently binding proteins to solid matrices are known in the art. The nature of the solid surface will vary depending upon the assay format. For assays carried out in microtiter wells, the solid surface will be the wall of the well or cup. For assays using beads, the solid surface will be the surface of the bead. In assays using a dipstick (i.e., a solid body made from a porous or fibrous material such as fabric or paper) the surface will be the surface of the material from which the dipstick is made. In agglutination assays the solid surface may be the surface of latex or gelatin particles. When individual antigens are bound to the matrix they may be distributed homogeneously on the surface or distributed thereon in a pattern, such as bands so that a pattern of antigen binding may be discerned.

Simple mixtures of the antigens comprise the antigens in any suitable solvent or dispersing medium.

Assay Formats Using Combinations of Antigens

The HCV antigens may be employed in virtually any assay format that employs a known antigen to detect antibodies. A common feature of all of these assays is that the antigen is contacted with the body component suspected of containing HCV antibodies under conditions that permit the antigen to bind to any such antibody present in the component. Such conditions will typically be physiologic temperature, pH and ionic strength using an excess of antigen. The incubation of the antigen with the specimen is followed by detection of immune complexes comprised of the antigen.

Design of the immunoassays is subject to a great deal of variation, and many formats are known in the art. Protocols may, for example, use solid supports, or immunoprecipitation. Most assays involve the use of labeled antibody or polypeptide; the labels may be, for example, enzymatic, fluorescent, chemiluminescent, radio-active, or dye molecules. Assays which amplify the signals from the immune complex are also known; examples of which are assays which utilize biotin and avidin, and enzyme-labeled and mediated immunoassays, such as ELISA assays.

The immunoassay may be, without limitation, in a heterogeneous or in a homogeneous format, and of a standard or competitive type. In a heterogeneous format, the polypeptide is typically bound to a solid matrix or support to facilitate separation of the sample from the polypeptide after incubation. Examples of solid supports that can be used are nitrocellulose (e.g., in membrane or microtiter well form), polyvinyl chloride (e.g., in sheets or microtiter wells), polystyrene latex (e.g., in beads or microtiter plates, polyvinylidene fluoride (known as Immulon™), diazotized paper, nylon membranes, activated beads, and Protein A beads. For example, Dynatech Immulon™ 1 or Immulon™ 2 microtiter plates or 0.25 inch polystyrene beads (Precision Plastic Ball) can be used in the heterogeneous format. The solid support containing the antigenic polypeptides is typically washed after separating it from the test sample, and prior to detection of bound antibodies. Both standard and competitive formats are known in the art.

In a homogeneous format, the test sample is incubated with the combination of antigens in solution. For example, it may be under conditions that will precipitate any antigen-antibody complexes which are formed. Both standard and competitive formats for these assays are known in the art.

In a standard format, the amount of HCV antibodies forming the antibody-antigen complex is directly monitored. This may be accomplished by determining whether labeled anti-xenogenic (e.g., anti-human) antibodies which recognize an epitope on anti-HCV antibodies will bind due to complex formation. In a competitive format, the amount of HCV antibodies in the sample is deduced by monitoring the competitive effect on the binding of a known amount of labeled antibody (or other competing ligand) in the complex.

Complexes formed comprising anti-HCV antibody (or, in the case of competitive assays, the amount of competing antibody) are detected by any of a number of known techniques, depending on the format. For example, unlabeled HCV antibodies in the complex may be detected using a conjugate of anti-xenogenic Ig complexed with a label, (e.g., an enzyme label).

In an immunoprecipitation or agglutination assay format the reaction between the HCV antigens and the antibody forms a network that precipitates from the solution or suspension and forms a visible layer or film of precipitate. If no anti-HCV antibody is present in the test specimen, no visible precipitate is formed.

The HCV antigens will typically be packaged in the form of a kit for use in these immunoassays. The kit will normally contain in separate containers the combination of antigens (either already bound to a solid matrix or separate with reagents for binding them to the matrix), control antibody formulations (positive and/or negative), labeled antibody when the assay format requires same and signal generating reagents (e.g., enzyme substrate) if the label does not generate a signal directly. Instructions (e.g., written, tape, VCR, CD-ROM, etc.) for carrying out the assay usually will be included in the kit.

The following examples are intended to illustrate the invention and are not intended to limit the invention

in any manner.

Example 1: Synthesis of HCV Antigen C33c

6 HCV antigen C33c contains a sequence from the NS3 domain. Specifically, it includes amino acids 1192-1457 of Figure 1. This antigen was produced in bacteria as a fusion protein with human superoxide dismutase (SOD) as follows. The vector pSODcf1 (Steiner et al. (1986), J. Virol. 58:9) was digested to completion with EcoRI and BamHI and the resulting EcoRI, BamHI fragment was ligated to the following linker to form pcf1EF:

10 GATC CTG GAA TTC TGA TAA
GAC CTT AAG ACT ATT TTA A

A cDNA clone encoding amino acids 1192-1457 and having EcoRI ends was inserted into pcf1EF to form pcf1EF/C33c. This expression construct was transformed into D1210 E. coli cells.

15 The transformants were used to express a fusion protein comprised of SOD at the N-terminus and in-frame C33c HCV antigen at the C-terminus. Expression was accomplished by inoculating 1500 ml of Luria broth containing ampicillin (100 micrograms/ml) with 15 ml of an overnight culture of the transformants. The cells were grown to an O.D. of 0.3, IPTG was added to yield a final concentration of 2 mM, and growth continued until the cells attained a density of 1 O.D., at which time they were harvested by centrifugation at 3,000 x g at 4°C for 20 minutes. The packed cells can be stored at -80°C for several months.

In order to purify the SOD-C33c polypeptide the bacterial cells in which the polypeptide was expressed were subjected to osmotic shock and mechanical disruption, the insoluble fraction containing SOD-C33c was isolated and subjected to differential extraction with an alkaline-NaCl solution, and the fusion polypeptide in the extract purified by chromatography on columns of S-Sepharose and Q-Sepharose.

25 The crude extract resulting from osmotic shock and mechanical disruption was prepared by the following procedure. One gram of the packed cells were suspended in 10 ml of a solution containing 0.02 M Tris HCl, pH 7.5, 10 mM EDTA, 20% sucrose, and incubated for 10 minutes on ice. The cells were then pelleted by centrifugation at 4,000 x g for 15 min at 4°C. After the supernatant was removed, the cell pellets were resuspended in 10 ml of Buffer A1 (0.01M Tris HCl, pH 7.5, 1 mM EDTA, 14 mM beta-mercaptoethanol [BME]), and incubated on ice for 10 minutes. The cells were again pelleted at 4,000 x g for 15 minutes at 4°C. After removal of the clear supernatant (periplasmic fraction I), the cell pellets were resuspended in Buffer A1, incubated on ice for 10 minutes, and again centrifuged at 4,000 x g for 15 minutes at 4°C. The clear supernatant (periplasmic fraction II) was removed, and the cell pellet resuspended in 5 ml of Buffer A2 (0.02 M Tris HCl, pH 7.5, 14 mM BME, 1 mM EDTA, 1 mM PMSF). In order to disrupt the cells, the suspension (5 ml) and 7.5 ml of Dyno-mill lead-free acid washed glass beads (0.10-0.15 mm diameter) (obtained from Glen-Mills, Inc.) were placed in a Falcon tube, and vortexed at top speed for two minutes, followed by cooling for at least 2 min on ice; the vortexing-cooling procedure was repeated another four times. After vortexing, the slurry was filtered through a scintered glass funnel using low suction; the glass beads were washed two times with Buffer A2, and the filtrate and washes combined.

40 The insoluble fraction of the crude extract was collected by centrifugation at 20,000 x g for 15 min at 4°C, washed twice with 10 ml Buffer A2, and resuspended in 5 ml of MILLI-Q water.

A fraction containing SOD-C33c was isolated from the insoluble material by adding to the suspension NaOH (2 M) and NaCl (2 M) to yield a final concentration of 20 mM each, vortexing the mixture for 1 minute, centrifuging at 20,000 x g for 20 min at 4°C, and retaining the supernatant.

45 In order to purify SOD-C33c on S-Sepharose, the supernatant fraction was adjusted to a final concentration of 6M urea, 0.05M Tris HCl, pH 7.5, 14 mM BME, 1 mM EDTA. This fraction was then applied to a column of S-Sepharose Fast Flow (1.5 x 10 cm) which had been equilibrated with Buffer B (0.05M Tris HCl, pH 7.5, 14 mM BME, 1 mM EDTA). After application, the column was washed with two column volumes of Buffer B. The flow through and wash fractions were collected. The flow rate of application and wash, was 1 ml/min; and collected fractions were 1 ml. In order to identify fractions containing SOD-C33c, aliquots of the fractions were analyzed by electrophoresis on 10% polyacrylamide gels containing SDS followed by staining with Coomassie blue. The fractions are also analyzable by Western blots using an antibody directed against SOD. Fractions containing SOD-C33c were pooled.

55 Further purification of SOD-C33c was on a Q-Sepharose column (1.5 x 5 cm) which was equilibrated with Buffer B. The pooled fractions containing SOD-C33c obtained from chromatography on S-Sepharose was applied to the column. The column was then washed with Buffer B, and eluted with 60 ml of a gradient of 0.0 to 0.4 M NaCl in Buffer B. The flow rate for application, wash, and elution was 1 ml/min; collected fractions

were 1 ml. All fractions from the Q-Sepharose column were analyzed as described for the S-Sepharose column. The peak of SOD-C33c eluted from the column at about 0.2 M NaCl.

The SOD-C33c obtained from the Q-Sepharose column was greater than about 90% pure, as judged by analysis on the polyacrylamide SDS gels and immunoblot using a monoclonal antibody directed against human SOD.

Example 2: Synthesis of HCV Antigen C100

HCV antigen C100 contains sequences from the NS3 and NS4 domains. Specifically, it includes amino acids 1569-1931 of Figure 1. This antigen was produced in yeast. A cDNA fragment of a 1270 bp encoding the above amino acids and heaving EcoRI termini was prepared.

The construction of a yeast expression vector in which this fragment was fused directly to the *S. cerevisiae* ADH2/GAP promoter was accomplished by a protocol which included amplification of the C100 sequence using a PCR method, followed by ligation of the amplified sequence into a cloning vector. After cloning, the C100 sequence was excised, and with a sequence which contained the ADH2/GAP promoter, was ligated to a large fragment of a yeast vector to yield a yeast expression vector.

The PCR amplification of C100 was performed using as template the vector pS3-56_{C100m}, which had been linearized by digestion with Sall. pS3-56, which is a pBR322 derivative, contains an expression cassette which is comprised of the ADH2/GAPDH hybrid yeast promoter upstream of the human superoxide dismutase gene, and a downstream alpha factor transcription terminator.

The oligonucleotide primers used for the amplification were designed to facilitate cloning into the expression vector, and to introduce a translation termination codon. Specifically, novel 5'-HindIII and 3'-Sall sites were generated with the PCR oligonucleotides. The oligonucleotide containing the Sall site also encodes the double termination codons, TAA and TGA. The oligonucleotide containing the HindIII site also contains an untranslated leader sequence derived from the pgap63 gene, situated immediately upstream of the AUG codon. The pEco63GAPDH gene is described by Holland and Holland (1980) and by Kniskern et al. (1986). The PCR primer sequences used for the direct expression of C100m were:

5' GAG TGC TCA AGC TTC AAA ACA AAA TGG CTC
ACT TTC TAT CCC AGA CAA AGC AGA GT 3'

and

5' GAG TGC TCG TCG ACT CAT TAG GGG GAA
ACA TGG TTC CCC CGG GAG GCG AA 3'.

Amplification by PCR, utilizing the primers, and template, was with a Cetus-Perkin-Elmer PCR kit, and was performed according to the manufacturer's directions. The PCR conditions were 29 cycles of 94°C for a minute, 37°C for 2 minutes, 72°C for 3 minutes; and the final incubation was at 72°C for 10 minutes. The DNA can be stored at 4°C or -20°C overnight.

After amplification, the PCR products were digested with HindIII and Sall. The major product of 1.1 kb was purified by electrophoresis on a gel, and the eluted purified product was ligated with a large Sall-HindIII fragment of pBR322. In order to isolate correct recombinants, competent HB101 cells were transformed with the recombinant vectors, and after cloning, the desired recombinants were identified on the basis of the predicted size of HindIII-Sall fragments excised from the clones. One of the clones which contained the a HindIII-Sall fragment of the correct size was named pBR322/C100^d. Confirmation that this clone contained amplified C100 was by direct sequence analysis of the HindIII-Sall fragment.

The expression vector containing C100 was constructed by ligating the HindIII-Sall fragment from pBR322/C100^d to a 13.1 kb BamHI-Sall fragment of pBS24.1, and a 1369 bp BamHI-HindIII fragment containing the ADH2/GAP promoter. (The latter fragment is described in EPO 164,556). The pBS24.1 vector is described in commonly owned U.S.S.N. 382,805 filed 19 July 1989. The ADH2/GAP promoter fragment was obtained by digestion of the vector pGAP/AG/HindIII with HindIII and BamHI, followed by purification of the 1369 bp fragment on a gel.

Competent HB101 cells were transformed with the recombinant vectors; and correct recombinants were identified by the generation of a 2464 bp fragment and a 13.1 kb fragment generated by BamHI and Sall diges-

tion of the cloned vectors. One of the cloned correct recombinant vectors was named pC100-d#3.

In order to express C100, competent cells of *Saccharomyces cerevisiae* strain AB122 (MATa leu2 ura3-53 prb 1-1122 pep4-3 prc1-407[cir-0]) were transformed with the expression vector pC100-d#3. The transformed cells were plated on URA-sorbitol, and individual transformants were then streaked on Leu⁻ plates.

Individual clones were cultured in Leu⁻, ura⁻ medium with 2% glucose at 30°C for 24-36 hours. One liter of Yeast Extract Peptone Medium (YEP) containing 2% glucose was inoculated with 10 ml of the overnight culture, and the resulting culture was grown at 30°C at an agitation rate of 400 rpm and an aeration rate of 1 L of air per 1 L of medium per minute (i.e., 1vvm) for 48 hours. The pH of the medium was not controlled. The culture was grown in a BioFlo II fermentor manufactured by New Brunswick Science Corp. Following fermentation, the cells were isolated and analyzed for C100 expression.

Analysis for expressed C100 polypeptide by the transformed cells was performed on total cell lysates and crude extracts prepared from single yeast colonies obtained from the Leu⁻ plates. The cell lysates and crude extracts were analyzed by electrophoresis on SDS polyacrylamide gels, and by Western blots. The Western blots were probed with rabbit polyclonal antibodies directed against the SOD-C100 polypeptide expressed in yeast. The expected size of the C100 polypeptide is 364 amino acids. By gel analysis the expressed polypeptide has a MW, of 39.9K.

Both analytical methods demonstrated that the expressed C100 polypeptide was present in total cell lysates, but was absent from crude extracts. These results suggest that the expressed C100 polypeptide may be insoluble.

Example 3: Expression of HCV Antigen S2

HCV antigen S2 contains a sequence from the hydrophobic N-terminus of the S domain. It includes amino acids 199-328 of Figure 1.

The protocol for the construction of the expression vector encoding the S2 polypeptide and for its expression in yeast was analogous to that used for the expression of the C100 polypeptide, described in Example 2.

The template for the PCR reaction was the vector pBR322/Pi14a, which had been linearized by digestion with HindIII. Pi14a is a cDNA clone that encodes amino acids 199-328.

The oligonucleotides used as primers for the amplification by PCR of the S2 encoding sequence were the following.

For the 5'-region of the S2 sequence:

5' GAG TGC TCA AGC TTC AAA ACA AAA TGG GGC TCT
ACC ACG TCA CCA ATG ATT GCC CTA AC 3';

and

for the 3'-region of the S2 sequence:

5' GAG TGC TCG TCG ACT CAT TAA GGG GAC CAG TTC
ATC ATC ATA TCC CAT GCC AT 3'.

The primer for the 5'-region introduces a HindIII site and an ATG start codon into the amplified product. The primer for the 3'-region introduces translation stop codons and a SalI site into the amplified product.

The PCR conditions were 29 cycles of 94°C for a minute, 37°C for 2 minutes, 72°C for 3 minutes, and the final incubation was at 72°C for 10 minutes.

The main product of the PCR reaction was a 413 bp fragment, which was gel purified. The purified fragment was ligated to the large fragment obtained from pBR322 digested with HindIII and SalI fragment, yielding the plasmid pBR322/S2d.

Ligation of the 413 bp HindIII-SalI S2 fragment with the 1.36 kb BamHI-HindIII fragment containing the ADH2/GAP promoter, and with the large BamHI-SalI fragment of the yeast vector pBS24.1 yielded recombinant vectors, which were cloned. Correct recombinant vectors were identified by the presence of a 1.77 kb fragment after digestion with BamHI and SalI. An expression vector constructed from the amplified sequence, and containing the sequence encoding S2 fused directly to the ADH2/GAP promoter is identified as pS2d#9.

Example 4: Synthesis of HCV C Antigen

HCV antigen C22 is from the C domain. It comprises amino acids 1-122 of Figure 1.

The protocol for the construction of the expression vector encoding the C polypeptide and for its expression in yeast was analogous to that used for the expression of the C100 polypeptide, described supra, except for the following.

The template for the PCR reaction was pBR322/ Ag30a which had been linearized with HindIII. Ag30 is a cDNA clone that encodes amino acids 1-122. The oligonucleotides used as primers for the amplification by PCR of the C encoding sequence were the following.

For the 5'-region of the C sequence:

5' GAG TGC AGC TTC AAA ACA AAA TGA GCA CGA
ATC CTA AAC CTC AAA AAA AAA AC 3',

and

for the 3'-region of the C sequence:

5' GAG TGC TCG TCG ACT CAT TAA CCC AAA TTG CGC
GAC CTA CGC CGG GGG TCT GT 3'.

The primer for the 5'-region introduces a HindIII site into the amplified product, and the primer for the 3'-region introduces translation stop codons and a SalI site. The PCR was run for 29 cycles of 94°C for a minute, 37°C for 2 minutes, 72°C for 3 minutes, and the final incubation was at 72°C for 10 minutes.

The major product of PCR amplification is a 381 bp polynucleotide. Ligation of this fragment with the SalI-HindIII large SalI-HindIII fragment of pBR322 yielded the plasmid pBR322/C2.

Ligation of the 381 bp HindIII-SalI C coding fragment excised from pBR322/C2 with the 1.36 kb BamHI-HindIII fragment containing the ADH2/GAP promoter, and with the large BamHI-SalI fragment of the yeast vector pBS24.1 yielded recombinant vectors, which were cloned. Correct recombinant vectors were identified by the presence of a 1.74 kb fragment after digestion with BamHI and SalI. An expression vector constructed from the amplified sequence, and containing the sequence encoding C fused directly to the ADH2/GAP promoter is identified as pC22.

Analysis for expressed C polypeptide by the transformed cells was performed on total cell lysates and crude extracts prepared from single yeast colonies obtained from the Leu⁻ plates. The cell lysates and crude extracts were analyzed by electrophoresis on SDS polyacrylamide gels. The C polypeptide is expected to have 122 amino acids and by gel analysis the expressed polypeptide has a MW, of approximately 13.6 Kd.

Example 5: Synthesis of NS5 Polypeptide

This polypeptide contains sequence from the N-terminus of the NS5 domain. Specifically it includes amino acids 2054 to 2484 of Figure 1. The protocol for the construction of the expression vector encoding the NS5 polypeptide and for its expression were analogous to that used for the expression of C33c (see Example 1).

Example 6: Radioimmunoassay (RIA) for Antibodies to HCV

The HCV antigens of Examples 1-5 were tested in an RIA format for their ability to detect antibodies to HCV in the serum of individuals clinically diagnosed as having HCV (Non-A, Non-B) and in serum from blood given by paid blood donors.

The RIA was based upon the procedure of Tsu and Herzenberg (1980) in SELECTED METHODS IN CELLULAR IMMUNOLOGY (W.H. Freeman & Co.), pp. 373-391. Generally, microtiter plates (Immulon 2, Removawell strips) are coated with purified HCV antigen. The coated plates are incubated with the serum samples or appropriate controls. During incubation, antibody, if present, is immunologically bound to the solid phase antigen. After removal of the unbound material and washing of the microtiter plates, complexes of human antibody-NANBV antigen are detected by incubation with ¹²⁵I-labeled sheep anti-human immunoglobulin. Unbound labeled antibody is removed by aspiration, and the plates are washed. The radioactivity in individual wells is

determined; the amount of bound human anti-HCV antibody is proportional to the radioactivity in the well.

Specifically, one hundred microliter aliquots containing 0.1 to 0.5 micrograms of the HCV antigen in 0.125 M Na borate buffer, pH 8.3, 0.075 M NaCl (BBS) was added to each well of a microtiter plate (Dynatech Immulon 2 Removawell Strips). The plate was incubated at 4°C overnight in a humid chamber, after which, the antigen solution was removed and the wells washed 3 times with BBS containing 0.02% Triton X-100 (BBST). To prevent non-specific binding, the wells were coated with bovine serum albumin (BSA) by addition of 100 microliters of a 5 mg/ml solution of BSA in BBS followed by incubation at room temperature for 1 hour; after this incubation the BSA solution was removed. The antigens in the coated wells were reacted with serum by adding 100 microliters of serum samples diluted 1:100 in 0.01M Na phosphate buffer, pH 7.2, 0.15 M NaCl (PBS) containing 10 mg/ml BSA, and incubating the serum containing wells for 1 hr at 37°C. After incubation, the serum samples were removed by aspiration, and the wells were washed 5 times with BBST. Antibody bound to the antigen was determined by the binding of ¹²⁵I-labeled F(ab)₂ sheep anti-human IgG to the coated wells. Aliquots of 100 microliters of the labeled probe (specific activity 5-20 microcuries/microgram) were added to each well, and the plates were incubated at 37°C for 1 hour, followed by removal of excess probe by aspiration, and 5 washes with BBST. The amount of radioactivity bound in each well was determined by counting in a counter which detects gamma radiation.

Table 1 below presents the results of the tests on the serum from individuals diagnosed as having HCV.

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Table 1

	<u>INDIVIDUAL</u>	<u>ANTIGEN</u>				
		<u>S2</u>	<u>C22</u>	<u>C100</u>	<u>C33c</u>	<u>NS5</u>
5	CVH IVDA	P	P	P(+++)	P	P
	CVH IVDA	P	P	P(+)	P	P
	CVH IVDA	P	P	P(+)	P	P
10	CVH NOS	P	P	P	P	P
	AVH NOS HS	N	N	N	N	N
	AVH NOS HS	P	N	N	N	N
15	AVH NOS HS	P	N	N	N	N
	AVH NOS HS	P/N	N	N	N	N
	AVH PTVH	N	N	N	P/N	N
	AVH NOS HS	N	N	N	N	N
20	AVH NOS	N	N	N	N	P
	AVH PTVH	N	N	N	N	N
	AVH IVDA	N	P	N	P	P
25	AVH PTVH	P	P/N	N	N	P
	AVH NOS	N	P	N	N	N
	AVH IVDA	N	P	N	P	P
	AVH NOS HS	P/N	N	N	N	N
30	AVH PTVH	N	N	N	N	N
	CVH IVDA	P	P	P	P	P
	CVH IVDA	P	P	P	P	P
	AVH NOS HS	N	N	N	N	N
35	CVH PTVH	P	P	N	N	N
	AVH PTVH	P	N	P(+)	P(+++)	N
	CVH PTVH	N	P	P	P	P
40	CVH NOS HS	P	P	P	P	N
	CVH NOS	N	P	P/N	P	P

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<u>INDIVIDUAL</u>		<u>ANTIGEN</u>			
		<u>S2</u>	<u>C22</u>	<u>C100</u>	<u>C33c</u> <u>NS5</u>
5	CVH IVDA	N	N	N	P N
	AVH IVDA	P	P	P	P P
	AVH IVDA	P	P	P	P P
10	CVH IVDA	P	P	P	P P
	AVH IVDA	P/N	P	N	P P
	AVH IVDA	N	P	P	P N
15	CVH PTVH	P	P/N	N	N N
	CVH NOS	N	N	N	N N
	CVH NOS	N	N	N	N N
20	CVH IVDA	P	P	P	P P
	AVH IVDA	P	P	P	P P
	CVH PTVH	P	P	P	P P
25	AVH PTVH?	N	P	P	P P
	AVH IVDA	N	P	N	P N
	AVH NOS	N	N	N	N N
30	AVH NOS	N	N	N	N N
	CVH NOS	N	P	N	N P
	CVH NOS	P	P	N	N N
35	CVH NOS HS	P	P	P	P P
	CVH PTVH	P	P	N	P P
	AVH nurse	P	P	N	N N
40	AVH IVDA	P	P	P	P N
	AVH IVDA	N	P	P(+)	P(+++) N
	AVH nurse	P/N	P	N	N N
45	AVH PTVH	P/N	P	P	N P
	AVH NOS	N	P/N	N	N P
	AVH NOS	N	P	N	P N
50	AVH PTVH	P	P/N	N	N N
	AVH PTVH	N	P	N	P P
	AVH PTVH	P	P	P	P P
55	AVH PTVH	N	P	N	N P
	CVH PTVH	P/N	P	P(+)	P(+++) N
	AVH PTVH	N	P/N	P(+)	P(+++) P

<u>INDIVIDUAL</u>		<u>ANTIGEN</u>			
		<u>S2</u>	<u>C22</u>	<u>C100</u>	<u>C33c</u> <u>NS5</u>
	AVH PTVH	P	(?)	P	N P
5	CVH PTVH	N	P	N	P P
	CVH PTVH	N	P	P	P P
	CVH PTVH	N	N	N	N N
10	AVH NOS	N	N	N	N N
	AVH nurse	P	P	N	N N
	CVH PTVH	N	P	N	N P
	AVH IVDA	N	P	N	P/N N
15	CVH PTVH	P	P	P(+)	P(+++) P
	AVH NOS	P	P	N	N N
	AVH NOS	P/N	P	N	N P
	AVH PTVH	P/N	P	P	P P
20	AVH NOS	N	P	P	P P/N
	AVH IVDA	N	P	N	N P
	AVH NOS	N	P/N	N	N N
25	AVH NOS	P	P	N	N P
	AVH PTVH	N	P	P	P P
	crypto	P	P	P	P P
	CVH NOS	N	P	P	P P
30	CVH NOS	N	N	N	N N
	AVH PTVH	N	P	P(+)	P(++) N
	AVH PTVH	N	P/N	P(+)	P(++) P
35	AVH PTVH	N	P/N	P(+)	P(++) P
	CVH IVDA	P	P	P	P P
	CVH IVDA	P	P	P	P P
	CVH IVDA	P	P	P	P P
40	CVH IVDA	P	P	P	P P
	AVH NOS	N	P	N	N N
	CVH IVDA	P	P	P	P P/N
	AVH IVDA	P	P	P	P N
45	AVH NOS	P	P	N	N N
	AVH NOS	P	P	N	N N
	CVH PTVH	P	P	N	N P/N

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<u>INDIVIDUAL</u>		<u>ANTIGEN</u>			
		<u>S2</u>	<u>C22</u>	<u>C100</u>	<u>C33c</u> <u>NS5</u>
	AVH PTVH	N	P	N	P P
5	AVH NOS	N	N	N	N N
	AVH NOS	N	P	N	N N
	AVH NOS	P	N	N	N N
10	CVH NOS	N	N	N	N N
	AVH NOS	N	P/N	N	N N
	AVH IVDA	N	P	P	P P
	crypto	N	P	N	N P/N
15	crypto	P	P	P/N	P P
	AVH IVDA	N	N	P	P N
	AVH IVDA	N	P	P	P N
	AVH NOS	N	N	N	N N
20	AVH NOS	N	N	N	N N
	CVH IVDA	P	P	P	P P
	CVH PTVH	N	N	N	N N
25	CVH PTVH	P	P	P(+)	P(+++) P
	CVH PTVH	P	P	P(+)	P(+++) P
	CVH NOS	P/N	N	N	N N
	CVH NOS	N	N	N	N N
30	CVH PTVH	P	P	P	P P
	CVH PTVH	P	P	P	P P
	CVH PTVH	P	P	P	P P
35	AVH IVDA	N	P	P	P P
	CVH NOS	N	N	N	N N
	CVH NOS	N	N	N	N N
	CVH PTVH	P	P	P	P P
40	AVH NOS	P	P	N	N P/N
	AVH NOS	N	P/N	N	N N
	CVH PTVH	P	P	N	N P
	CVH NOS	N	P/N	N	N N
45	AVH NOS	N	P	N	N N
	AVH NOS	N	P	N	N N
	CVH PTVH	N	P	N	N N

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<u>INDIVIDUAL</u>		<u>ANTIGEN</u>			
		<u>S2</u>	<u>C22</u>	<u>C100</u>	<u>C33c</u> <u>NS5</u>
5	AVH IVDA	N	P	N	P P
	AVH NOS	P	N	N	N N
	CVH NOS	N	N	N	N N
	CVH NOS	N	N	N	N N
10	CVH IVDA	P	P	P	P P
	CVH IVDA	P/N	P	P	P P
	CVH IVDA	P	P	P	P P
	CVH IVDA	N	P	P	P P
15	AVH NOS	N	P	N	N N
	CVH IVDA	N	P	N	N P
	CVH IVDA	N	P	N	N P
	AVH PTVH	P	P	N	P P
20	AVH PTVH	P	P	N	P P
	CVH NOS	N	P/N	N	N P/N
	CVH NOS	N	P	N	N N
	CVH NOS	N	N	N	N N
25	CVH PTVH	P	P	P	P P
	CVH PTVH	P	P	P	P P
	CVH PTVH	P	P	P	P P
	AVH IVDA	N	P	N	N P
30	AVH IVDA	N	P	P(++)	P(+) P
	CVH PTVH	P	P	P	P P
	AVH PTVH	N	P	P	P P
	CVH PTVH?	N	P	P	P P
35	CVH PTVH?	P/N	P	P	P P
	CVH NOS HS	P	P	N	N N
	CVH IVDA	P	P	P	P N
	CVH PTVH	N	P	P	P P
40	CVH PTVH	P	P	P	P P/N
	CVH NOS	P	P	P	P P
	CVH IVDA	P	P	P	P P
	CVH PTVH	P	P	P	P N
45	CVH PTVH	P	P	P	P P
	CVH PTVH	P	P	P	P P
	CVH PTVH	P	P	P	P P
	CVH PTVH	P	P	P	P P

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<u>INDIVIDUAL</u>		<u>ANTIGEN</u>			
		<u>S2</u>	<u>C22</u>	<u>C100</u>	<u>C33c</u> <u>NS5</u>
5	CVH NOS	N	N	N	N P/N
	CVH NOS	N	P/N	N	N P/N
	CVH PTVH	P	P	P	P P
10	CVH NOS	N	P	N	N N
	CVH NOS	N	N	N	N N
	CVH NOS	P	P	N	N P/N
	CVH NOS	N	N	N	N N
	CVH NOS HS	P	P	P	P P
15	CVH NOS HS	P	P	P	P P
	CVH PTVH	N	N	N	N N
	AVH PTVH	N	P	P	P P
	AVH NOS			-	-
20	CVH PTVH	N	P	P/N	P(+++) N
	crypto	P	P	P	P P
	crypto	P	P	P	P P
	crypto	N	P	N	N N
25	crypto	N	P	P	P P
	CVH PTVH	P	P	P	P P
	crypto	N	N	N	N N
	crypto	N	P	N	N P/N
30	crypto	N	P	N	N P
	crypto	P	P	P	P P
	crypto	N	P	N	P N
	crypto			-	-
35	crypto			-	-
	crypto			-	-
	CVH NOS			-	-
	AVH-IVDA	N	P	N	P(+) P

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INDIVIDUALANTIGEN

	<u>S2</u>	<u>C22</u>	<u>C100</u>	<u>C33c</u>	<u>NS5</u>
AVH-IVDA	N	P/N	N	P(++)	N

AVH = acute viral hepatitis

CVH = chronic viral hepatitis

PTVH = post-transfusion viral hepatitis

IVDA = intravenous drug abuser

crypto = cryptogenic hepatitis

NOS = non-obvious source

P = positive

N = negative

Per these results, no single antigen reacted with all sera. C22 and C33c were the most reactive and S2 reacted with some sera from some putative acute HCV cases with which no other antigen reacted. Based on these results, the combination of two antigens that would provide the greatest range of detection is C22 and C33c. If one wished to detect a maximum of acute infections, S2 would be included in the combination.

Table 2 below presents the results of the testing on the paid blood donors.

Table 2

		<u>Antigens</u>				
	<u>Donor</u>	<u>C100</u>	<u>C33c</u>	<u>C22</u>	<u>S2</u>	<u>NS5</u>
30	1	N	N	N	N	N
	2	N	N	N	N	N
	3	P	P	P	P	P
35	4	N	N	N	N	N
	5	N	N	N	N	N
	6	N	N	N	N	N
	7	N	N	N	N	N
40	8	N	N	N	N	N

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		Antigens				
	<u>Donor</u>	<u>C100</u>	<u>C33c</u>	<u>C22</u>	<u>S2</u>	<u>NS5</u>
5	9	N	N	N	N	N
	10	N	N	N	N	N
	11	N	N	N	N	N
	12	N	N	N	N	N
10	13	N	N	N	N	N
	14	N	N	N	N	N
	15	N	N	N	N	N
	16	N	N	N	N	N
15	17	N	N	N	N	N
	18	P	P	P	P	P
	19	P	P	N	P	P
	20	P	P	N	P	P
20	21	N	N	N	N	N
	22	N	P	P	N	P
	23	P	P	P	P	P
	24	N	N	N	N	N
25	25	N	N	N	N	N
	26	N	N	N	N	N
	27	N	N	N	N	N
	28	N	N	N	N	N
30	29	N	N	N	N	N
	30	N	N	N	N	N
	31	P	P	P	N	P
	32	N	N	N	N	N
35	33	N	N	N	N	N
	34	N	N	N	N	P
	35	N	N	P	N	P
	36	N	N	N	N	N
40	37	N	N	N	N	N
	38	N	N	N	N	N
	39	N	N	N	N	N
	40	N	N	N	N	N
45	41	N	N	N	N	P
	42	N	N	N	N	N

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Antigens						
	<u>Donor</u>	<u>C100</u>	<u>C33c</u>	<u>C22</u>	<u>S2</u>	<u>NS5</u>
5	43	N	N	N	N	N
	44	N	N	N	N	N
	45	N	N	N	N	N
	46	N	N	N	N	N
10	47	P	P	N	N	P
	48	N	N	N	N	N
	49	N	N	N	N	N
	50	N	N	N	N	N
15	51	N	P	P	N	P
	52	N	N	N	N	N
	53	N	P	P	N	P
	54	P	P	P	P	N
20	55	N	N	N	N	N
	56	N	N	N	N	N
	57	N	N	N	N	N
	58	N	N	N	N	N
25	59	N	N	N	N	N
	60	N	N	N	N	N
	61	N	N	N	N	N
	62	N	N	N	N	N
30	63	N	N	N	N	N
	64	N	N	N	N	N
	65	N	N	N	N	N
	66	N	N	N	N	N
35	67	N	N	N	N	N
	68	N	N	N	N	N
	69	N	N	N	N	N
	70	P	P	P	P	P
40	71	N	N	N	N	N
	72	N	N	N	N	N
	73	P	P	P	P	N
	74	N	N	N	N	N
45	75	N	N	N	N	N
	76	N	N	N	N	P

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Antigens						
	<u>Donor</u>	<u>C100</u>	<u>C33c</u>	<u>C22</u>	<u>S2</u>	<u>NS5</u>
5	77	N	N	N	N	N
	78	N	N	N	N	N
	79	N	N	N	N	N
	80	N	N	N	N	N
10	81	N	N	N	N	N
	82	N	N	N	N	N
	83	P	P	N	N	N
	84	N	N	P	N	N
15	85	N	N	N	N	N
	86	P	P	P	P	N
	87	N	N	N	N	N
	88	N	N	N	N	N
20	89	P	P	P	P	P
	90	P	P	P	P	N
	91	N	N	N	N	P
	92	P	P	P	N	N
25	93	N	N	N	N	N
	94	N	N	N	N	N
	95	N	N	N	N	N
	96	N	N	N	N	N
30	97	N	N	N	N	N
	98	N	P	P	P	P
	99	P	P	P	P	P
	100	N	N	N	N	N
35	101	P	P	P	P	P
	102	N	N	N	N	N
	103	N	N	N	N	N
	104		N	N	N	N
40	105	P	P	P	P	N
	106	N	N	N	N	N
	107	N	N	N	N	N
	108	N	N	N	N	N
45	109	P	P	P	P	P
	110	P	P	P	N	P

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		Antigens				
	<u>Donor</u>	<u>C100</u>	<u>C33c</u>	<u>C22</u>	<u>S2</u>	<u>NS5</u>
5	111	P	P	P	N	P
	112	N	N	N	N	N
	113	P	P	P	P	P
	114	N	N	N	N	N
10	115	N	N	N	N	N
	116	P	P	P	P	P
	117	N	N	N	N	N
	118	N	N	N	N	N
15	119	N	N	N	N	N
	120	P	P	P	P	P
	121	N	N	N	N	N
	122	N	P	P	N	P
20	123	N	N	N	N	N
	124	N	N	N	N	N
	125	N	N	N	N	N
	126	P	N	N	N	N
25	127	N	N	N	N	N
	128	N	N	N	N	N
	129	N	N	N	N	N
	130	P	P	P	P	N
30	131	N	N	N	N	P
	132	N	N	N	N	N
	133	N	N	N	N	N
	134	N	N	N	N	N
35	135	N	N	N	N	N
	136	N	N	N	N	N
	137	N	N	N	N	N
	138	N	N	N	N	N
40	139	N	N	N	N	N
	140	P	N	N	N	N
	141	P	N	P	P	P
	142	N	N	N	N	N
45	143	N	N	N	N	N
	144	N	N	N	N	N

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Antigens						
	<u>Donor</u>	<u>C100</u>	<u>C33c</u>	<u>C22</u>	<u>S2</u>	<u>NS5</u>
5	145	N	N	N	N	N
	146	N	N	N	N	N
	147	N	N	N	N	N
	148	N	N	N	N	N
10	149	N	N	N	N	N
	150	N	N	N	N	N
	151	N	N	N	N	N
	152	N	N	N	N	N
15	153	N	N	N	N	N
	154	P	P	P	P	P
	155	N	N	N	N	N
	156	N	N	N	N	N
20	157	N	N	N	N	N
	158	N	N	N	N	N
	159	N	N	N	N	N
	160	N	N	N	N	N
25	161	P	P	P	P	P
	162	N	N	N	N	N
	163	N	N	N	N	N
	164	P	P	P	N	P
30	165	N	N	N	N	N
	166	P	P	P	N	P
	167	N	N	N	N	N
	168	N	N	N	N	N
35	169	N	N	N	N	N
	170	N	N	N	N	N
	171	N	N	N	N	N
	172	N	N	N	N	N
40	173	N	N	N	N	N
	174	N	N	N	N	N
	175	N	N	N	N	N
	176	N	N	N	N	N
45	177	N	N	N	N	P
	178	N	N	N	N	N

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		Antigens				
	Donor	C100	C33c	C22	S2	NS5
5	179	N	N	N	N	N
	180	N	N	N	N	N
	181	N	N	N	N	N
	182	N	N	N	N	N
10	183	P	P	P	P	P
	184	N	N	N	N	N
	185	N	N	N	N	N
	186	N	N	N	N	N
15	187	N	N	N	N	N
	188	N	P	P	N	N
	189	N	N	N	N	N
	190	N	N	N	N	N
20	191	N	N	N	N	N
	192	N	N	N	N	N
	193	N	N	N	N	N
	194	N	N	N	N	N
25	195	N	N	N	N	N
	196	N	N	N	N	N
	197	N	N	N	N	P
	198	P	P	P	N	N
30	199	N	N	N	N	P
	200	P	P	P	P	N

35

The results on the paid donors generally confirms the results from the sera of infected individuals.

Example 7: ELISA Determinations of HCV Antibodies Using Combination of HCV Antigens

40 Plates coated with a combination of C22 and C33c antigens are prepared as follows. A solution containing coating buffer (50mM Na Borate, pH 9.0), 21 ml/plate, BSA (25 micrograms/ml), C22 and C33c (2.50 micrograms/ml each) is prepared just prior to addition to the Removeawell Immulon I plates (Dynatech Corp.). After mixing for 5 minutes, 0.2ml/well of the solution is added to the plates, they are covered and incubated for 2 hours at 37°C, after which the solution is removed by aspiration. The wells are washed once with 400 microliters wash buffer (100 mM sodium phosphate, pH 7.4, 140 mM sodium chloride, 0.1% (W/V) casein, 1% (W/V) Triton x-100, 0.01% (W/V) Thimerosal). After removal of the wash solution, 200 microliters/well of Postcoat solution (10 mM sodium phosphate, pH 7.2, 150 mM sodium chloride, 0.1% (w/v) casein, 3% sucrose and 2 mM phenylmethylsulfonylfluoride (PMSF)) is added, the plates are loosely covered to prevent evaporation, and are allowed to stand at room temperature for 30 minutes. The wells are then aspirated to remove the solution, and lyophilized dry overnight, without shelf heating. The prepared plates may be stored at 2-8°C in sealed aluminum pouches with dessicant (3 g Sorb-it™ packs).

50 In order to perform the ELISA determination, 20 microliters of serum sample or control sample is added to a well containing 200 microliters of sample diluent (100 mM sodium phosphate, pH 7.4, 500 mM sodium chloride, 1 mM EDTA, 0.1% (W/V) Casein, 0.01% (W/V) Thimerosal, 1% (W/V) Triton X-100, 100 micrograms/ml yeast extract). The plates are sealed, and are incubated at 37°C for two hours, after which the solution is removed by aspiration, and the wells are washed three times with 400 microliters of wash buffer (phosphate buffered saline (PBS) containing 0.05% Tween 20). The washed wells are treated with 200 microliters of mouse anti-human-IgG-horse radish peroxidase (HRP) conjugate contained in a solution of Ortho conjugate diluent

(10 mM sodium phosphate, pH 7.2, 150 mM sodium chloride, 50° (V/V) fetal bovine serum, 1% (V/V) heat treated horse serum, 1 mM $K_3Fe(CN)_6$, 0.05% (W/V) Tween 20, 0.02% (W/V) Thimerosal). Treatment is for 1 hour at 37°C, the solution is removed by aspiration, and the wells are washed three times with 400 ml wash buffer, which is also removed by aspiration. To determine the amount of bound enzyme conjugate, 200 microliters of substrate solution (10 mg O-phenylenediamine dihydrochloride per 5 ml of Developer solution) is added. Developer solution contains 50 mM sodium citrate adjusted to pH 5.1 with phosphoric acid, and 0.6 microliters/ml of 30% H_2O_2 . The plates containing the substrate solution are incubated in the dark for 30 minutes at room temperature, the reactions are stopped by the addition of 50 microliters/ml 4N sulfuric acid, and the ODs determined.

In a similar manner, ELISAs using fusion proteins of C22 and C33c, and C22, C33c, and S2 and combinations of C22 and C100, C22 and S2, C22 and an NS5 antigen, C22, C33c, and S2, and C22, C100, and S2 may be carried out.

Modifications of the above-described modes for carrying out the invention that are obvious to those of skill in the fields of molecular biology, immunology, and related fields are intended to be within the scope of the following claims.

Claims

1. A combination of synthetic hepatitis C viral (HCV) antigens comprising:
 - (a) a first HCV antigen from the C domain; and
 - (b) at least one additional HCV antigen selected from the group consisting of
 - (i) an HCV antigen from the NS3 domain;
 - (ii) an HCV antigen from the NS4 domain;
 - (iii) an HCV antigen from the S domain; and
 - (iv) an HCV antigen from the NS5 domain.
2. A combination of synthetic hepatitis C viral (HCV) antigens comprising:
 - (a) a first HCV antigen consisting essentially of the C domain; and
 - (b) a second HCV antigen from the NS3 domain.
3. The combination of claim 2 wherein the first HCV antigen is C22 and the second HCV antigen is C33c.
4. The combination of claim 2 including (c) a third HCV antigen from the S domain.
5. The combination of claim 3 including (c) HCV antigen S2.
6. A combination of synthetic HCV antigens comprising:
 - (a) a first HCV antigen consisting essentially of the C domain; and
 - (b) a second HCV antigen from the NS4 domain.
7. The combination of claim 6 wherein the first HCV antigen is C22 and the second HCV antigen is C100.
8. The combination of claim 6 including (c) a third HCV antigen from the S domain.
9. The combination of claim 7 including (c) HCV antigen S2.
10. The combination of claim 1, 2, 3, 4, 5, 6, 7, 8 or 9 wherein the combination is in the form of a fusion polypeptide.
11. The combination of claim 1, 2, 3, 4, 5, 6, 7, 8 or 9 wherein the combination is in the form of said first HCV antigen and said additional antigens individually bound to a common solid matrix.
12. The combination of claim 11 wherein the solid matrix is the surface of a microtiter plate well, a bead or a dipstick.
13. The combination of claim 1, 2, 3, 4, 5, 6, 7, 8 or 9 wherein the combination is in the form of a mixture of said first HCV antigen and said additional HCV antigen(s).

14. A method for detecting antibodies to hepatitis C virus (HCV) in a mammalian body component suspected of containing said antibodies comprising contacting said body component with the combination of synthetic HCV antigens of claim 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 under conditions that permit antibody-antigen reaction and detecting the presence of immune complexes of said antibodies and said antigens.

15. A method for detecting antibodies to hepatitis C virus (HCV) in a mammalian body component suspected of containing said antibodies comprising contacting said body component with a panel of synthetic HCV antigens comprising:

(a) a first HCV antigen from the C domain; and

(b) at least one additional HCV antigen selected from the group consisting of

(i) an HCV antigen from the NS3 domain;

(ii) an HCV antigen from the NS4 domain;

(iii) an HCV antigen from the S domain; and

(iv) an HCV antigen from the NS5 domain under conditions that permit antibody-antigen reaction and detecting the presence of immune complexes of said antibodies and said antigens.

16. A kit for carrying out an assay for detecting antibodies to hepatitis C antigen (HCV) in a mammalian body component suspected of containing said antibodies comprising in packaged combination:

(a) the combination of synthetic HCV antigens of any one of claims 1-13;

(b) standard control reagents; and

(c) instructions for carrying out the assay.

-341 GGCAGCCCCCTGATGGGGGCGA
CGGTGGGGGACTACCCCCGCT

-319 CACTCCACCATGAATCACTCCCTGTGAGGAACTACTGTCTTCACGCAGAAAGCGTCTAG
GTGAGGTGGTACTTAGTGAGGGGACACTCCTTGATGACAGAAGTGGCTCTTCGCAGATC

-259 CCATGGCGTTAGTATGAGTGTCTGTCAGCCTCCAGGACCCCCCTCCCGGGAGAGCCATA
GGTACCSCAATCATACTCACAGCACCTCGGAGGTCTCGGGGGGAGGGCCCTCTCGGTAT

-199 GTGGTCTCGGGAACCGGTGAGTACACCGGAATTGCCAGGACGACCGGGTCTTTCTTGGG
CACCAGACGCTTGCCCACTCATGTGGCCTTAACGGTCTCTGTCGCCAGGAAAGAACCT

-139 TCAACCCGCTCAATGCTGGAGATTGGGCGCTGCCCGCGAAGACTGCTAGCCGAGTAGT
AGTTGGGCGAGTTACGGACCTCTAAACCCGACGGGGGCGTTCTGACGATCGGCTCATCA

- 79 GTTGGGTCCGGAAGGCGCTTGTGGTACTGCCTGATAGGGTGGTTGCGAGTCCCCGGGAG
CAACCCAGCGCTTTCGGGAACACCATGACGGACTATCCACGAACGCTCACGGGGCCCTC

- 19 GTCTCGTAGACCGTGCAAC
CAGAGCATCTGGCACGTTGG

--- Arg Thr

MetSerThrAsnProLysProGlnLysLysAsnLysArgAsnThrAsnArgArgProGln
ATGAGCACGAATCCTAAACCTCAAAAAAAAAACAAACGTAACCAACCGTCCGCCACAG
TACTCGTGCTTAGGATTGGAGTTTTTTTTTTGTTGTCATTGTGGTTGGCAGCGGGTGTCT

61 AspValLysPheProGlyGlyGlyGlnIleValGlyGlyValTyrLeuLeuProArgArg
GACGTCAAGTTCCCGGTGGCGGTGAGATCGTTGGTGGAGTTACTTGTGTCGGCGCAGG
CTGCAGTTCAAGGGCCACCGCCAGTCTAGCAACCACCTCAATGAACACGCGCGCTCC

121 GlyProArgLeuGlyValArgAlaThrArgLysThrSerGluArgSerGlnProArgGly
GGCCCTAGATTGGGTGTGCGCGGACGAGAAAGACTTCGAGCGGTGCGAACCTCGAGGT
CCGGATCTAACCCACACGCGCGCTGCTCTTTCTGAAGGCTCGCCAGCGTTGGAGCTCCA

181 ArgArgGlnProIleProLysAlaArgArgProGluGlyArgThrTrpAlaGlnProGly
AGACGTCAGCCTATCCCAAGGCTCGTCCGCCCCAGGGGAGGACCTGGGCTCAGCCCGGG
TCTGCAGTCGGATAGGGGTTCCGAGCAGCCGGGCTCCCGTCTTGGACCGAGTCGGGCC

241 TyrProTrpProLeuTyrGlyAsnGluGlyCysGlyTrpAlaGlyTrpLeuLeuSerPro
TACCTTGGCCCCCTATGGCAATGAGGGCTGCGGGTGGGGGGGATGGCTCTGTCTCCC
ATCGGAACCGGGGACATACCGTTACTCCCGACGCCACCCGCTTACCGAGACAGAGGG

301 ArgGlySerArgProSerTrpGlyProThrAspProArgArgArgSerArgAsnLeuGly
CGTGGCTCTCGGCCCTAGCTGGGGCCCCACAGACCCCCGCGTAGGTGCGGCAATTTGGGT
GCACCTAGAGCCGGATCGACCTCGGGGTGTCTGGGGGCGCATCCAGCGCTTAAACCCA

361 LysValIleAspThrLeuThrCysGlyPheAlaAspLeuMetGlyTyrIleProLeuVal
AAGGTCATCGATACCTTACGTGCGGCTTCGCGACCTCATGGGGTACATACCGCTCGTC
TTCCASTAGCTATGGGAATGCACGCCGAAGCGGCTGGAGTACCCCATGTATGGCGAGCAG

421 GlyAlaProLeuGlyGlyAlaAlaArgAlaLeuAlaHisGlyValArgValLeuGluAsp
GGCGCTCTCTGGAGGCGCTGCCAGGGCCCTGGCGCATGCGGTCCGGTCTCTGGAAGAC
CCGCGGGGAGAACCTCCGCGACGCTCCCGGACCGCGTACCGCAGGCCAAGACCTTCTG

Thr

481 GlyValAsnTyrAlaThrGlyAsnLeuProGlyCysSerPheSerIlePheLeuLeuAla
GGCGTGAACATGCAACAGGGAATCTTCTGTTGCTCTTCTCTATCTTCTTCTGCCC
CCGCATTGATACGTTGTCTCTTGGAGGACCAACGAGAAAGAGATAGAAGCAAGACCGG

541 LeuLeuSerCysLeuThrValProAlaSerAlaTyrGlnValArgAsnSerThrGlyLeu
TGGCTCTCTTCTGACTGTGCGCGCTTGGGCTTACCAAGTGCGCACTCCACGGGGCTT
GACGAGAGAACGAACTGACACGGGCGAAGCCGATGGTTACGCGTTGAGGTGCCCTCGAA

601 TyrHisValThrAsnAspCysProAsnSerSerIleValTyrGluAlaAlaAspAlaIle
TACCAGTCAACCAATGATTGCCCTAACTCGASTATTGTGTACGAGGCGGCGGATGCCATC
ATGGTGCAGTGGTTACTAACGGGATTGAGCTATAACACATGCTCCGCGGGCTACGGTAG

Figure 1 (Sheet 1 of 10)

661 LeuHisThrProGlyCysValProCysValArgGluGlyAsnAlaSerArgCysTrpVal
 CTGCACACTTCGGGGTGGTCCCTTTCGTTTCGTGAGGGCAACGCTCGAGGTGTTCGGGTG
 GACGTGTGAGGGCCCCACGCAGGGAACGCAAGCACTCCCGTTGCGGAGCTCCACACCCAC
 721 AlaMetThrProThrValAlaThrArgAspGlyLysLeuProAlaThrGlnLeuArgArg
 SCGATGACCCCTACGGTGGCCACCAGGATGSCAACTCCCGCGACGCAGCTTCGACGT
 CGCTACTGGGGATGCCACCGGTGGTCCCTACCGTTTGAGGGGCGGTGCGTCAAGCTGCA
 781 HisIleAspLeuLeuValGlySerAlaThrLeuCysSerAlaLeuTyrValGlyAspLeu
 CACATCGATCTGCTTGTTCGGGAGCGCCACCCCTCTGTTGGGCCCTCTACGTGGGGGACCTA
 GTGTAGCTAGACGAACAGCCCTCGCGTGGGAGACAAGCCGGAGATGCACCCCTGGAT
 841 CysGlySerValPheLeuValGlyGlnLeuPheThrPheSerProArgArgHisTrpThr
 TGGGGTCTGTCTTCTTGTGCGCCAACTGTTCACCTTCTCTCCAGGCGCCACTGGACG
 ACGCCAGACAGAAAGAACAGTCGGTTGACAAGTGGAGAGAGGGTCCGCGGTGACCTGC
 901 ThrGlnGlyCysAsnCysSerIleTyrProGlyHisIleThrGlyHisArgMetAlaTrp
 ACGCAAGGTTGCAATTGCTCTATCTATCCCGCCATATAACGGGTACCCGATGGCATGG
 TCGCTTCCACGTTAACGAGATAGATAGGGCCGCTATATTGCCAGTGGCGTACCGTACC
 Val
 961 AspMetMetMetAsnTrpSerProThrThrAlaLeuValMetAlaGlnLeuLeuArgIle
 GATATCATGATGAACCTGGTCCCTACGACGGCGTTGGTAAATGGCTCAGCTGCTCCGGATC
 CTATACTACTACTTGACCAGGGGATGCTGCCGCAACCATTACCGAGTCGACGAGGCCCTAG
 1021 ProGlnAlaIleLeuAspMetIleAlaGlyAlaHisTrpGlyValLeuAlaGlyIleAla
 CCACAAGCCATCTTGGACATGATCGCTGGTGTCTCACTGGGGAGTCTTGGCGGGCATAGCG
 GGTGTTCCGGTAGAACCTGTACTAGCGACCACGAGTGACCCCTCAGGACCGCCCGTATCGC
 1081 TyrPheSerMetValGlyAsnTrpAlaLysValLeuValValLeuLeuPheAlaGly
 TATTTCTCCATGGTGGGAACTGSGCGAAGGTCTCTGGTAGTGTCTGTCTATTGCGCGG
 ATAAAGAGGTACCAACCCCTTGACCCGCTTCCAGGACCATCAGCAGCAGATAAACGGCCG
 1141 ValAspAlaGluThrHisValThrGlyGlySerAlaGlyHisThrValSerGlyPheVal
 GTCGACSCGGAACCCACGTACCCGGGGGAAGTGCCGGCCACACTGTGTCTGGATTGTGT
 CAGCTGCGCCTTTGGGTGCAGTGGCCCCCTTCACGGCCGGTGTGACACAGACCTAAACAA
 1201 SerLeuLeuAlaProGlyAlaLysGlnAsnValGlnLeuIleAsnThrAsnGlySerTrp
 AGCCTCCTCGCACCAGGCGCCAAAGCAGAACGTCCAGCTGATCAACCAACGGCAGTTGG
 TCGGAGGAGCGTGCTCGCGGTTCGTCTTCAGGTGCGACTAGTTGTGGTTGCCGTCAACC
 1261 HisLeuAsnSerThrAlaLeuAsnCysAsnAspSerLeuAsnThrGlyTrpLeuAlaGly
 CACCTCAATAGCACGGCCCTGAACCTGCAATGATAGCCTCAACACCGGCTGGTTGGCAGGG
 GTGGAGTTATCGTGGCGGACTTGACGTTACTATCGGAGTTGTGGCCGACCAACGTTCC
 1321 LeuPheTyrHisHisLysPheAsnSerSerGlyCysProGluArgLeuAlaSerCysArg
 CTTTCTATCACCACAAGTCAACTTTCAGGCTGTCTCTGAGAGGCTAGCCAGCTGCCGA
 GAAAAGATAGTGGTGTTCAGTTCGAGAAGTCCGACAGGACTCTCCGATCGGTGCGAGGCT
 1381 ProLeuThrAspPheAspGlnGlyTrpGlyProIleSerTyrAlaAsnGlySerGlyPro
 CCCCTTACCGATTTTGACCAGGGCTGGGGCCCTATCAGTTATGCCAACGGAAGCGCCCC
 GGGGAATGGCTAAACCTGGTCCCGACCCCGGATAGTCAATACGGTTGCCTTCGCCGGGG
 1441 AspGlnArgProTyrCysTrpHisTyrProPheLysProCysGlyIleValProAlaLys
 GACCAGCGCCCTACTGCTGGCACTACCCCCAAACCTTGCGGTATTGTGCCCGCGAAG
 CTGGTCCGGGGATGACGACCGTGATGGGGGTTTGGAAACGCCATAACACGGGCGCTTC
 1501 SerValCysGlyProValTyrCysPheThrProSerProValValValGlyThrThrAsp
 AGTGTGTGTGCTCCGGTATTTGCTTCACTCCAGCCCCGTGGTGGTGGGAACGACCGAC
 TCACACACACAGGCCATATAACGAAGTGAGGGTGGGGCACCACCAACCTTGCTGGCTG
 1561 ArgSerGlyAlaProThrTyrSerTrpGlyGluAsnAspThrAspValPheValLeuAsn
 AGGTCCGGCGCGCCACCTACAGCTGGGGTGAATGATACGGACGCTTCTCGTCTTAAC
 TCCAGCCCGCGGGGTGGATGTGACCCCACTTTTACTATGCCTGCAGAAGCAGGAATTG
 AsnThrArgProProLeuGlyAsnTrpPheGlyCysThrTrpMetAsnSerThrGlyPhe

Figure 1 (Sheet 2 of 10)

162- AATACCAGGCCACCGCTGGGCAATTGGTTTCGGTTGTACCTGGATGAACCTCACTGGATTCT
 TATGGTCCGGTGGCGACCCGTTAACCAGCCACATGGACCTACTTGAGTIGACCTAAG

 ThrLysValCysGlyAlaProProCysValIleGlyGlyAlaGlyAsnAsnThrLeuHis
 1681 ACCAAGTGTGGGAGCGCTCTTGTGTCATCGGAGGGGCGGGCAACAACACCTGCGAC
 TGGTTTCACACGCTCGCGGAGGAACACAGTAGCCTCCCCGCCGCTGTGTGGGACGTG

 CysProThrAspCysPheArgLysHisProAspAlaThrTyrSerArgCysGlySerGly
 1741 TGCCCCACTGATTGCTTCCGCAAGCATCGGACGCCACATACTCTCGGTCCGCTCCGGT
 ACGGGGTGACTAACGAAGGCGTTCSTAGGCTTGGGTGTATGAGAGCCACGCCGAGGCCA

 Ile
 ProTrpLeuThrProArgCysLeuValAspTyrProTyrArgLeuTrpHisTyrProCys
 1801 CCTGGATCACACCCAGGTGCCCTGGTTCGACTACCCGTATAGCCTTTGGCATTATCCTTGT
 GGGACCTAGTGTGGGTCCACGGACCGCTGTATGGGCATATCCGAAACCGTATAGGAACA

 ThrIleAsnTyrThrIlePheLysIleArgMetTyrValGlyGlyValGluHisArgLeu
 1861 ACCATCAACTACACCATATTTAAATCAGGATGTACGTGGGAGGGGTGGAACACAGGCTG
 TGGIAGTTGATGTGGTATAAATTTTAGTCCATATGCACCTCCCCAGCTTGTGTCCGAC

 GluAlaAlaCysAsnTrpThrArgGlyGluArgCysAspLeuGluAspArgAspArgSer
 1921 GAAGCTGCCTGCAACTGGACGCGGGGCGAACGTTGCGATCTGGAAGACAGGACAGGTCC
 CTTGACGGACGTTACCTGCGCCCGCTTGCAACGCTAGACCTTCTGTCCGTGTCAGG

 GluLeuSerProLeuLeuLeuThrThrThrGlnTrpGlnValLeuProCysSerPheThr
 1981 GAGCTCAGCCGTTACTGCTGACCACTACACAGTGGCAGGTCTCCCGTGTTCCTTCACA
 CTCGAGTCGGGCAATGACGACTGGTGATGTCTACCGTCCAGGAGGGCACAAGGAAGTGT

 ThrLeuProAlaLeuSerThrGlyLeuIleHisLeuHisGlnAsnIleValAspValGln
 2041 ACCCTACCAGCCTTGTCCACCGGCTCATCCACCTCCACCAAGACATTGTGGACGTGCGAG
 TGGGATGGTGGGAACAGGTGGCCGAGTAGGTGGAGGTGGTCTTGTAACACCTGCACGTC

 TyrLeuTyrGlyValGlySerSerIleAlaSerTrpAlaIleLysTrpGluTyrValVal
 2101 TACTGTACGGGTGGGGTCAAGCATCGCGTCTCTGGGCCATTAAAGTGGGAGTACGTGCTT
 ATGAACATGCCCCACCCAGTTCTGTAGCGCAGGACCCGGTAATTCACCTCATGCAGCAA

 LeuLeuPheLeuLeuLeuAlaAspAlaArgValCysSerCysLeuTrpMetMetLeuLeu
 2161 CTCTGTCTCTTCTGCTTGACAGCGCGCGCTCTGCTCTCTGCTGTGTGGATGATGCTACTC
 GAGGACAAGGAAGACGAACGCTCTGCGCGCAGACGAGGACGAACACCTACTACGATGAG

 IleSerGlnAlaGluAlaAlaLeuGluAsnLeuValIleLeuAsnAlaAlaSerLeuAla
 2221 ATATCCCAAGCGGAGGCGGCTTGGAGAACCCTCGTAATACTTAATGCAGCATCCCTGGCC
 TATAGGGTTCCCTCCGCCGAAACCTCTTGGAGCATTATGAATTACGTCTGAGGGACCGG

 GlyThrHisGlyLeuValSerPheLeuValPhePheCysPheAlaTrpTyrLeuLysGly
 2281 GGGACGCACGGTCTGTATCCTTCTCTGCTTCTTCTGCTTTCATGGTATTGTAAGGGT
 CCTGCGTGCCAGAACATAGGAAGGAGCACAGAGAAGACGAACGTAACATAACTTCCCA

 LysTrpValProGlyAlaValTyrThrPheTyrGlyMetTrpProLeuLeuLeuLeuLeu
 2341 AAGTGGGTGCCCGGAGCGGTCTACACCTTCTACGGGATGTGGCCTCTCCTCCTGCTCCTG
 TTCACCCACGGGCTCGCCAGATGTGGAAGATGCCCTACACCGGAGAGGAGGACGAGGAC

 LeuAlaLeuProGlnArgAlaTyrAlaLeuAspThrGluValAlaAlaSerCysGlyGly
 2401 TTGGCGTTGCCCCAGCGGGCTACGCGCTGGACACGAGGTGGCCGCGTCTGTGGCGGT
 AACCCCAACGGGCTCGCCCGCATGCGCGACCTGTGCCCTCCACCGGCGACACACCGCCA

 ValValLeuValGlyLeuMetAlaLeuThrLeuSerProTyrTyrLysArgTyrIleSer
 2461 GTTGTCTCTGTCGGGTGATGGCGCTGACTCTGTACCATATTACAAGCGCTATATCAGC
 CAACAAGAGCAGCCCACTACCGCGACTGAGACAGTGGTATAATGTTCCGATATAGTCG

 (Asn)
 TrpCysLeuTrpTrpLeuGlnTyrPheLeuThrArgValGluAlaGlnLeuHisValTrp
 2521 TGGTGTCTGTGGTGGCTTCACTATTTCTGACCAGAGTGGAGCGCAACTGCACGTGTGG
 ACCACGAACACCCAGGTCATAAARGACTGGTCTCACCTTCGCGTTGACGTGCTACC

 IleProProLeuAsnValArgGlyGlyArgAspAlaValIleLeuLeuMetCysAlaVal

Figure 1 (Sheet 3 of 10)

2581 ATTCCCCCCCCCAACGTCGAGGGGGGGGGACGGCCGTCATCTTACTCATGTGTGCTGTA
 TAAGGGGGGGAGTTGCAGGCTCCCCCGCGCTGCGGCAGTAGAATGAGTACACACGACAT
 HisProThrLeuValPheAspIleThrLysLeuLeuLeuAlaValPheGlyProLeuTrp
 2641 CACCCGACTCTGGTATTTGACATCACCAAATTGCTGCTGGCCGCTTCGGACCCCTTTGG
 CTGGGCTGAGACCATAAACTGTAGTGGTTTAAAGACGACCGGCAGAAAGCCTGGGGAAC
 IleLeuGlnAlaSerLeuLeuLysValProTyrPheValArgValGlnGlyLeuLeuArg
 2701 ATTCTTCAAGCCAGTTTGGCTAAAGTACCCTACTTTGTGGCGCTCAAGGCCTTCTCCGG
 TAAGAAGTTCGGTCAAACGAATTCATGGGATGAACACGCGCAGTTCCGGAAGAGGCC
 PheCysAlaLeuAlaArgLysMetIleGlyGlyHisTyrValGlnMetValIleIleLys
 2761 TCTGCGCGTTAGCGCGGAAGATGATCGGAGGCCATTACGTGCAATGGTCATCATTAG
 AAGACGCGCAATCGCGCCTTCTACTAGCTCCTGGTAAATGCAGTGTACCAAGTAGTAATTC
 LeuGlyAlaLeuThrGlyThrTyrValTyrAsnHisLeuThrProLeuArgAspTrpAla
 2821 TTAGGGGGCGCTTACTGGCAGCTATGTTTATAACCATCTCACTCCTCTTCGGGACTGGGCG
 AATCCCCCGGAATGACTGTGGATACAAATATTGGTAGAGTGAGGAGAAGCCCTGACCCCGC
 HisAsnGlyLeuArgAspLeuAlaValAlaValGluProValValPheSerGlnMetGlu
 2881 CACAACGGCTTTCGAGATCTGGCCGTGGCTGTAGAGCCAGTCTCTTCTCCCAAATGGAG
 GTGTGGCGAACGCTCTAGACCGGCACCGACATCTCGGTACAGCAGAAGAGGGTTTACCTC
 ThrLysLeuIleThrTrpGlyAlaAspThrAlaAlaCysGlyAspIleIleAsnGlyLeu
 2941 ACCAAGCTCATCAGTGGGGGGCAGATACCGCCGCGTGGGTGACATCATCAACGCGCTTG
 TGGTTCGAGTAGTGCACCCCGCTCTATGGCGCGCACGCCACTGTAGTAGTTGCCGAAC
 ProValSerAlaArgArgGlyArgGluIleLeuLeuGlyProAlaAspGlyMetValSer
 3001 CCTGTTTCCGCCCGCAGGGCGCGGAGATACTGCTCGGGCCAGCCGATGGAATGCTCTCC
 GGACAAAGGCGGGCGTCCCCGGCCTCTATGACGAGCCCGTGGCTACCTTACCAGAGG
 LysGlyTrpArgLeuLeuAlaProIleThrAlaTyrAlaGlnGlnThrArgGlyLeuLeu
 3061 AAGGGGTGGAGGTTGCTGGCGCCATCACGGCGTACGCCAGCAGACAAGGGGCTCTCTA
 TTCCCCACCTCCAACGACCGCGGGTAGTGCCCGCATGCGGGTCTGTCTTCCCCGAGGAT
 GlyCysIleIleThrSerLeuThrGlyArgAspLysAsnGlnValGluGlyGluValGln
 3121 GGGTGATAATCACCAGCCTAACTGGCCGGGACAAAACCAAGTGGAGGGTGAGGTCCAG
 CCCACGTATTAGTGGTTCGGATTGACCGGCCCTGTCTTTTGGTTCACTCCCACTCCAGGTC
 IleValSerThrAlaAlaGlnThrPheLeuAlaThrCysIleAsnGlyValCysTrpThr
 3181 ATTGTCTCACTGCTGCCCAAACCTTCTGGCAACGTGCATCAATGGGGGTGTCTGGACT
 TAACACAGTIGACGACGGGTTTGAAGGACCGTTGCAGTAGTTACCCCAACGACCTGA
 ValTyrHisGlyAlaGlyThrArgThrIleAlaSerProLysGlyProValIleGlnMet
 3241 GTCTACACGGGGCGGGAACGAGGACCATCGCGTCACCAAGGGTCTGTCTATCCAGATG
 CAGATGGTCCCCCGGCTTGTCTCTGGTAGCGAGTGGGTTCACAGGACAGTAGGTCTAC
 Ser Thr
 3301 TyrThrAsnValAspGlnAspLeuValGlyTrpProAlaProGlnGlySerArgSerLeu
 TATACCAATGTAGACCAGACCTTGTGGGCTGGCCCGCTCCGCAAGGTAGCCGCTCATTG
 ATATGTTTACATCTGGTTCTGGAACACCCGACCGGGCGAGGCGTTCCATCGGCGAGTAAC
 ThrProCysThrCysGlySerSerAspLeuTyrLeuValThrArgHisAlaAspValIle
 3361 ACACCCGTGCACTTGGCGCTCTCTCGACCTTTACCTGGTCACGAGGCACGCCGATGTCATT
 TGTGGGACGTGAACCGCGAGGAGCTTGAAGGACCGTGTGACGTCTCCGTGCGGCTACAGTAA
 ProValArgArgArgGlyAspSerArgGlySerLeuLeuSerProArgProIleSerTyr
 3421 CCGGTGCGCGGGCGGGGTGATAGCAGGGGCGAGCCTGCTGTGCGCCCGGCGCCATTTCCTAC
 GGGCAGCGGGCGGCCCACTATCTCCCCGTGCGACGACAGCGGGGCGGGGTAAGGATG
 LeuLysGlySerSerGlyGlyProLeuLeuCysProAlaGlyHisAlaValGlyIlePhe
 3481 ITGAAAGGCTCCTCGGGGGGTCCGCTGTGTGTCGGCGGGGCGGCGGCGGTCATATTT
 AACTTTCCGAGGAGCCCCCAGGCGACAACACGGGGCGCCCCGTGCGGCGACCCGTATAAA
 ArgAlaAlaValCysThrArgGlyValAlaLysAlaValAspPheIleProValGluAsn
 3541 AGGGCCCGGGTGTGCACCCGTGGAGTGGCTAAGGCGGTGGACTTTATCCCTGTGGAGAAC

TCCCGCGCCACACGTGGGCACCTCACCAGATTCGCGCCACCTGAAATAGGGACACCTCTTG
 3601 LeuGluThrThrMetArgSerProValPheThrAspAsnSerSerProProValValPro
 CTAGAGACAACCATCAGGTCCCCGGTGTTCACGGATAACTCTCTCCACCAGTAGTGCCC
 GATCTCTGTGGTACTCCAGGGGCCACAAGTGCCTATTGAGGAGAGGTGGTCATCAGGGG
 3661 GlnSerPheGlnValAlaHisLeuHisAlaProThrGlySerGlyLysSerThrLysVal
 CAGAGTTCCAGGTGGCTCACCTCCATGCTCCACAGGCAGCGGCAAAAGCACCAAGGTG
 GTCTGSAAGGTCCACCGAGTGGAGGTACGAGGGTGTCCGTGCGCGTTCCTCGTGTCCAG
 3721 ProAlaAlaTyrAlaAlaGlnGlyTyrLysValLeuValLeuAsnProSerValAlaAla
 CCGGCTGCATATGCAGCTCAGGGCTATAAGGTGCTAGTACTCAACCCCTCTGTGTGCTGCA
 GGCCGACGTATACGTGAGTCCCAGATATCCACGATCATGAGTTGGGGAGACAACGACGT
 3781 ThrLeuGlyPheGlyAlaTyrMetSerLysAlaHisGlyIleAspProAsnIleArgThr
 ACACTGGGCTTGGTGTCTACATGTCCAAGGCTCATGGATCGATCTCAACACTCAGGACC
 TGTGACCCGAAACCAGAAATGTACAGGTCCGAGTACCTAGCTAGGATTGTAGTCTCTGG
 3841 GlyValArgThrIleThrThrGlySerProIleThrTyrSerThrTyrGlyLysPheLeu
 GGGGTGAGAACAATTACCACTGGCAGCCCCATCAGTACTCCACCTACGGCAAGTTCCTT
 CCCCCTCTGTGTAATGGTGACCGTCCGGGTAGTGCATGAGGTGGATGCCGTCAAGGAA
 3901 AlaAspGlyGlyCysSerGlyGlyAlaTyrAspIleIleIleCysAspGluCysHisSer
 GCGGACGGCGGGTGTCTCGGGGGGCGCTTATGACATAATAATTGTGACGAGTGCCACTCC
 CGGCTCCGCCCCACGAGCCCCCGCGAATACTGTATTATTAAACACTGTCTCAGGTGAGG
 (Val)
 3961 ThrAspAlaThrSerIleLeuGlyIleGlyThrValLeuAspGlnAlaGluThrAlaGly
 ACGGATGCCACATCCATCTTGGGCATCGGCACTGTCTTGACCAAGCAGAGACTGCGGGG
 TGCTTACGGTGTAGGTAGAACCCCTAGCCGTGACAGGAAGTGGTTCGTCTGACGCCCC
 4021 AlaArgLeuValValLeuAlaThrAlaThrProProGlySerValThrValProHisPro
 GCGAGACTGGTGTCTCGCCACCGCCACCCCTCGGGCTCCGTCACTGTGCCCCATCCC
 CGCTCTGACCAACACGAGCGGTGGCGGTGGGGAGGCCGAGGCAGTGACACGGGGTAGGG
 4081 AsnIleGluGluValAlaLeuSerThrThrGlyGluIleProPheTyrGlyLysAlaIle
 AACATCGAGGAGGTGCTCTGTCCACCACGGAGAGATCCCTTTTACGGCAAGGCTATC
 TTGTAGCTCTCCACGAGACAGGTGGTGGCCTCTTAGGGAAAAATGCCGTTCGATAG
 4141 ProLeuGluValIleLysGlyGlyArgHisLeuIlePheCysHisSerLysLysLysCys
 CCCCTCGAAGTAATCAAGGGGGGAGACATCTCATCTTCTGTCAATCAAGAAGAAGTGC
 GGGGAGCTTCATTAGTTCCCCCTCTGTAGAGTAGAAGACASTAAGTTCTTCTTCACG
 4201 AspGluLeuAlaAlaLysLeuValAlaLeuGlyIleAsnAlaValAlaTyrTyrArgGly
 GACGAACCTGCGGCAAGCTGGTTCGCAATGGGCATCAATGCCCTGGCTACTACCGCGT
 CTGCTTGAGCGGCGTTTCGACCAGCGTAACCGTAGTTACGGCACCAGGATGATGGGCCA
 4261 LeuAspValSerValIleProThrSerGlyAspValValValValAlaThrAspAlaLeu
 CTGACGTGTCCCTCATCCGACCAGCGGCGATGTTGTGCTCTGGCAACCGATGCCCTC
 GAACTGCACAGGCAGTAGGGCTGGTCCCGCTACACAGCAGCACCGTTGGCTACGGGAG
 4321 MetThrGlyTyrThrGlyAspPheAspSerValIleAspCysAsnThrCysValThrGln
 ARGACCGGCTATACCGGCGACTTCGACTCGGGGATAGACTGCAATACGTGTGTACCCAG
 TACTGGCCGATATGGCCGCTGAAGCTGAGCCACTATCTGACGTTATGCACACAGTGGGT
 (Ser)
 4381 ThrValAspPheSerLeuAspProThrPheThrIleGluThrIleThrLeuProGlnAsp
 ACAGTCGATTTACGCCTTGACCTACCTTCACCATGAGACAATCAGCTCCCCCAGGAT
 TGTGAGCTAAAGTCGGAAGTGGATGGAAGTGGTAAGTCTGTTAGTGCGAGGGGGTCCCTA
 4441 AlaValSerArgThrGlnArgArgGlyArgThrGlyArgGlyLysProGlyIleTyrArg
 GCTGTCTCCCGCACTCAACGTGCGGGCAGGACTGGCAGGGGGGAAGCCAGGCATGAGA
 CGACAGAGGGCGTGAGTTGCAGCCCCGTCTGACCGTCCCCCTTCGGTCCGTAGATCTCT

PheValAlaProGlyGluArgProSerGlyMetPheAspSerSerValLeuCysGluCys
 4501 TTTGTGGCACCAGGGGAGCGCCCTCCGGCATGTTGACTCGTCCGTCCTCTGTGAGTGC
 AAACACCGTGGCCCTCGCGGGAGGGCCGTACAACTGAGCAGGCAGGAGACACTCAGC

 TyrAspAlaGlyCysAlaTrpTyrGluLeuThrProAlaGluThrThrValArgLeuArg
 4561 TATGACGCAGGCTGTGCTTGGTATGAGCTCAGCCCCCGGAGACTACAGTTAGGCTACGA
 ATACTCGTCCGACACGAACCATCTCGAGTGCAGGCGGGCTCTGATGTCAATCCGATGCT

 AlaTyrMetAsnThrProGlyLeuProValCysGlnAspHisLeuGluPheTrpGluGly
 4621 GCGTACATGAACACCCCGGGGCTTCCCGTGTGCCAGGACCATCTTGAATTTTGGGAGGGC
 CGCATGTACTTGTGGGGCCCCGAGGGCACACGGTCTTGGTAGAACTTAAACCTCCCG

 ValPheThrGlyLeuThrHisIleAspAlaHisPheLeuSerGlnThrLysGlnSerGly
 4681 GTCTTIACAGGCCTCACTCATATAGATGCCCACTTTCTATCCAGACAAAGCAGAGTGGG
 CAGAAATGTCCGGAGTGAGTATCTACGGGTGAAAGATAGGGTCTGTTTCGTCTCACC

 GluAsnLeuProTyrLeuValAlaTyrGlnAlaThrValCysAlaArgAlaGlnAlaPro
 4741 GAGAACCTTCTTACCTGGTAGCGTACCAAGCCACCGTGTGCGCTAGGGCTCAAGCCCCCT
 CTCTTGAAGGAATGGACCATCGCATGGTTCGGTGGCACACGGCATCCCGAGTTCGGGGA

 ProProSerTrpAspGlnMetTrpLysCysLeuIleArgLeuLysProThrLeuHisGly
 4801 CCCCCATCGTGGGACCGAGATGTGGAAGTGTTGATTGCGCTCAAGCCACCCCTCCATGGG
 GGGGGTAGCACCTCGTCTACACCTTCACAACTAAGCGGAGTTCGGGTGGGAGGTACCC

 ProThrProLeuLeuTyrArgLeuGlyAlaValGlnAsnGluIleThrLeuThrHisPro
 4861 CCAACACCCCTGCTATACAGACTGGGCGCTGTTGAGAATGAAATCACCCCTGAGCGACCCA
 GGTGTGGGGACGATATGTCTGACCCGCGACAAGTCTTACTTTAGTGGGACTGCGTGGST

 ValThrLysTyrIleMetThrCysMetSerAlaAspLeuGluValValThrSerThrTrp
 4921 GTCACCAATACATCATGACATGCTATGTCGGCCGACCTGGAGGTGCTCAGGAGCACCTGG
 CAGTGGTTATGTAGTACTGTACGTACAGCCGGCTGGACCTCCAGCAGTGTCTGTCGAC

 ValLeuValGlyGlyValLeuAlaAlaLeuAlaAlaTyrCysLeuSerThrGlyCysVal
 4981 GTGCTCGTGGCGGGCTCCTGGCTGCTTTGGCCGCGTATTGCTGTCAACAGGCTGCGTG
 CACGAGCAACCGCCGAGGACCGACGAAACCGGGCATAACGGACAGTTGTCCGACGCAC

 ValIleValGlyArgValValLeuSerGlyLysProAlaIleIleProAspArgGluVal
 5041 GTCATAGTGGGCAGGCTCGTCTTGTCCGGGAAGCCGGCAATCATACCTGACAGGGAAGTC
 CAGTATCACCCGTCCTCAGCAGAACAGGCCCTCGGCCGTAGTATGGACTGTCTCCTTCAG

 LeuTyrArgGluPheAspGluMetGluGluCysSerGlnHisLeuProTyrIleGluGln
 5101 CTCTACGAGAGTTGATGAGATGAAGAGTGTCTCAGCACTTACCGTACATCGAGCAA
 GAGATGGCTCTCAAGTACTCTACCTTCTCAGGAGATCGTGAATGGCATGGCTCGTT

 GlyMetMetLeuAlaGluGlnPheLysGlnLysAlaLeuGlyLeuGlnThrAlaSer
 5161 GGGATGATGCTCGCCGAGCAGTTCAAGCAGAGGCCCTCGGCCCTCTGACAGCCGCTCC
 CCTATACGAGCGCTCGTCAAGTTCGTCTTCCGGGAGCCGAGGACGTCTGGCGCAGG

 ArgGlnAlaGluValIleAlaProAlaValGlnThrAsnTrpGlnLysLeuGluThrPhe
 5221 CGTCAGGCAGAGGTTATCGCCCCCTGCTGTCCAGACCAACTGGCAAAACTCGAGACCTTC
 GCACTCCGTCTCCAATAGCGGGGACGACAGGTCTGGTTGACCGTTTTTGTAGCTCTGGAAG

 TrpAlaLysHisMetTrpAsnPhelIleSerGlyIleGlnTyrLeuAlaGlyLeuSerThr
 5281 TGGGCGAAGCATATGTGGAACCTTATCAGTGGGATACAATACTTGGCGGGCTTGTCAACG
 ACCCGCTTCGTATACACCTTGAAGTAGTCACCTATGTATGAACCGCCCGAACGTTGC

 LeuProGlyAsnProAlaIleAlaSerLeuMetAlaPheThrAlaAlaValThrSerPro
 5341 CTGCTTGGTAACCCCGCCATTGCTTCATTGATGGCTTTTACAGCTGCTGTACACGCCCA
 GACCGACCATTTGGGGGGTAACGAAGTAACCTACCGAAAATGTGACGACAGTGGTGGGT

 LeuThrThrSerGlnThrLeuLeuPheAsnIleLeuGlyGlyTrpValAlaAlaGlnLeu
 5401 CTAACCACTAGCCAAACCTCTCTTCAACATATTGGGGGGGTGGGTGGCTGCCAGCTC
 GATTGGTGATCGTTTGGGAGGAGAGTTGTATAACCCCCCACCACCGACGGGTCCAG

 AlaAlaProGlyAlaAlaThrAlaPheValGlyAlaGlyLeuAlaGlyAlaAlaIleGly
 5461 GTTGGGCTCGTGTGCTACTGCTTGTGGGCGCTGGCTTAGCTGGCGCCGCCATCGGC

Figure 1 (Sheet 6 of 10)

CGGCGGGGGCCACGGCGATGACGGAAACACCCGGGACCGAATCGACCGCGGGCGGTAGCCG
 SerValGlyLeuGlyLysValLeuIleAspIleLeuAlaGlyTyrGlyAlaGlyValAla
 5521 AGTGTGGACTGGGGAAGGTCTCTATAGACATCCTTGCAGGGTATGGCGCGGGCGTGGCG
 TCACAACTGACCCCTTCCAGGAGTATCTGTAGGAACGTCCCATACCGCGCCCGCACCGC
 (Gly)
 GlyAlaLeuValAlaPheLysIleMetSerGlyGluValProSerThrGluAspLeuVal
 5581 GGAGCTCTTGTGGCATTCAAGATCATGAGCGGTGAGGTCCCTCCACGGAGGACCTGGTC
 CCTCGAGAACACCGTAAGTTCTAGTACTCGCCACTCCAGGGGAGGTGCCTCCTGGACCAG
 AsnLeuLeuProAlaIleLeuSerProGlyAlaLeuValValGlyValValCysAlaAla
 5641 AATCTACTGCCCGCCATCTCTCGCCCGGAGCCCTCGTAGTCGGCGTGGTCTGTGCAGCA
 TTAGATGACGGGCGGTAGGAGAGCGGGCCTCGGGAGCATCAGCCGCACCGACACGTCGT
 IleLeuArgArgHisValGlyProGlyGluGlyAlaValGlnIrpMetAsnArgLeuIle
 5702 ATACTGCGCCCGCACGTGGCCCGGGCGAGGGGGCAGTGCAGTGGATGAACCGGCTGATA
 TATGACGGGCGGTGCAACCGGGCCCGCTCCCGCTCAGCTCACCTACTTGGCCGACTAT
 AlaPheAlaSerArgGlyAsnHisValSerProThrHisTyrValProGluSerAspAla
 5761 GCCTTCGCCTCCCGGGGGAACCATGTTTCCCCACGCACTACGTGCCGGAGAGCGATGCA
 CSGAAGCGGAGGGCCCCCTTGGTACAAAGGGGGTGCGTGATGCACGGCCTCTCGCTACGT
 (HisCys)
 AlaAlaArgValThrAlaIleLeuSerSerLeuThrValThrGlnLeuLeuArgArgLeu
 5821 GCTGCCCGCGTCACTGCCATACTCAGCAGCCTCACTGTAACCCAGCTCCTGAGGGGACTG
 CGACGGGCGCAGTGACGGTATGAGTCGTGAGTGACATTGGGTGAGGACTCCGCTGAC
 HisGlnTrpIleSerSerGluCysThrThrProCysSerGlySerTrpLeuArgAspIle
 5881 CACCASTGGATAAGCTCGGAGTGATCCACTCCATGCTCCGGTTCCTGGCTAAGGGACATC
 GTGGTCACCTATTGAGCCTCACATGGTGAGGTACGAGGCCAAGGACCGATTCCCTGTAG
 TrpAspTrpIleCysGluValLeuSerAspPheLysThrTrpLeuLysAlaLysLeuMet
 5941 TGGGACTGGATATGCGAGGTGTGAGCGACTTTAAGACCTGGCTAAAAGCTAAGCTCATG
 ACCCTGACCTATACGCTCCACAACCTCGCTGAAATCTTGACCGATTTCGATTGAGTAC
 ProGlnLeuProGlyIleProPheValSerCysGlnArgGlyTyrLysGlyValTrpArg
 6001 CCACAGCTGCCTGGGATCCCTTTGTGTCTGCCAGCGGGGTATAAGGGGGTCTGGCGA
 GGTGTCGACGGACCTAGGGGAAACACAGGACGGTCCGCGCCCATATTCCCCAGACCGCT
 (Val)
 GlyAspGlyIleMetHisThrArgCysHisCysGlyAlaGluIleThrGlyHisValLys
 6061 GTGGACGGCATCATGCACACTCGCTGCCACTGTGGAGCTGAGATCACTGGACATGTCAAA
 CACCTGCCSTAGTACGTGTGAGCGACGGTGACACCTCGACTCTAGTGACCTGTACAGTTT
 AsnGlyThrMetArgIleValGlyProArgThrCysArgAsnMetTrpSerGlyThrPhe
 6121 AACGGGACGATGAGGATCTCGGTCTTAGGACCTGCAGGAACATGTGGAGTGGGACCTTC
 TTGCCCTGCTACTCCTAGCAGCCAGGATCCTGGACGTCTTGTACACCTCACCTCGGAAG
 ProIleAsnAlaTyrThrThrGlyProCysThrProLeuProAlaProAsnTyrThrPhe
 6181 CCCATTAAATGCCATACACACGGGCCCCCTGTACCCCCCTTCTGCGCCGAACCTACAGCTTC
 GGGTAATTACGGATGTGGTGCCTGGGGGACATGGGGGGAAGGACGGGCTTGATGTGCAAG
 AlaLeuTrpArgValSerAlaGluGluTyrValGluIleArgGlnValGlyAspPheHis
 6241 CGGCTATGGAGGGTGTCTGCAGAGGAATATGTGGAGATAAGGCAGGTGGGGGACTTCCAC
 CGGATACCTCCACAGACGTCTCTTATACACCTCTATTCCGTCCACCCCTGAAGGTG
 TyrValThrGlyMetThrThrAspAsnLeuLysCysProCysGlnValProSerProGlu
 6301 TACGTGACGGGTATGACTACTGACAATCTCAAAATGCCCGTCCAGGTCCCATCGCCCGAA
 ATGCACTGCCCATACTGATGACTGTAGAGTTTACGGGCACGGTCCAGGGTAGCGGGCTT
 PhePheThrGluLeuAspGlyValArgLeuHisArgPheAlaProProCysLysProLeu
 6361 TTTTTCACAGAAATGGACGGGGTGGCGCTACATAGGTTTGGCGCCCCCTGCAAGCCTTTC
 AAAAAGTGTCTTAACCTGCCCCACGGGATGTATCCAAACGGGGGGGACGTTCCGGGAAC
 LeuArgGluGluValSerPheArgValGlyLeuHisGluTyrProValGlySerGlnLeu

Figure 1 (Sheet 7 of 10)

6421 CTGCGGJAGGAGGTATCATTAGASTAGGACTCCAGAAIACCCGGTAGGGTCGCRATTA
GACGCCCTCCTCCATAGTAAGTCTCATCCTGAGGTGCTTATGGGCATCCCAGCGTTAAT

6481 ProCysGluProGluProAspValAlaValLeuThrSerMetLeuThrAspProSerHis
CCTTGCGAGCCCGAACCGGACGTGGCGGTGTTGACGTCCATGCTCACTGATCCCTCCCAT
GGAACGCTCGGGCTTGGCCTGCACCGGCACAACTGCAGGTACGAGTGACTAGGGAGGGTA

6541 IleThrAlaGluAlaAlaGlyArgArgLeuAlaArgGlySerProProSerValAlaSer
ATAACAGCAGAGGCGGCGGGCGAAGTTGGCGAGGGGATCACCCCTCTGTGGCCAGC
TATTGTCGTCTCGGCCGCGCGCTTCCAACCGCTCCCTAGTGGGGGGAGACACCGGTG

6601 SerSerAlaSerGlnLeuSerAlaProSerLeuLysAlaThrCysThrAlaAsnHisAsp
TCCTCGGCTAGCCAGCTATCCGCTCCATCTCTCAAGGCACTTGACCGCTAACCATGAC
AGGAGCCGATCGGTGATAGGCGAGGTAGAGAGTTCGTTGAACGTGGCGATTGGTACTG

6661 SerProAspAlaGluLeuIleGluAlaAsnLeuLeuTrpArgGlnGluMetGlyGlyAsn
TCCCCTGATGCTGAGCTCATAGAGGCCAACCTCCTATGGAGGCAGGAGATGGCGGGCAAC
AGGGGACTACGACTCGAGTATCTCGGTTGGAGGATACCTCCGTCCTCTACCCGCGGTTG

6721 IleThrArgValGluSerGluAsnLysValValIleLeuAspSerPheAspProLeuVal
ATCACCAGGGTTGAGTCAGAAAACAAAGTGGTGATTCTGGACTCCTTCGATCCGCTTG
TAGTGGTCCCACTCAGTCTTTGTTTCACCACTAAGACCTGAGGAAGCTAGGCGAACAC

6781 AlaGluGluAspGluArgGluIleSerValProAlaGluIleLeuArgLysSerArgArg
GCGGAGGAGGACGAGCGGGAGATCTCCGTACCCGAGAAATCTGCGGAAGTCTCGGAGA
CGCTCTCTCTGCTCGCCCTCTAGAGGCATGCGCGTCTTTAGACGCTTCAGAGCCTCT

6841 PheAlaGlnAlaLeuProValTrpAlaArgProAspTyrAsnProProLeuValGluThr
TTCGCCCAGGCCCTGCCGTTTGGGCGCGGCCGACTATAACCCCCGCTAGTGGAGACG
AAGCGGCTCCGGGACGGGCAACCCGCGCGGCTGATATTGGGGGGCGATCACCTCTGC

6901 TrpLysLysProAspTyrGluProProValValHisGlyCysProLeuProProProLys
TGGAAAAGCCCCACTACGAACCACTGTGGTCCATGGCTGTCCGCTTCCACCTCCAAG
ACCTTTTGGGGCTGATGCTTGGTGGACACCAGGTACCGACAGGCGAAGGTGGAGGTTTC

6961 SerProProValProProProArgLysLysArgThrValValLeuThrGluSerThrLeu
TCCCTCTCTGCTCTCCGCTCGGAAGAAGCGGACGCTGGTCTCACTGAATCAACCCTA
AGGGGAGGACACGGAGGCGGAGCCTTCTTCCCTGCCACCGAGGTGACTTAGTTGGGAT

(Ser)

7021 SerThrAlaLeuAlaGluLeuAlaThrArgSerPheGlySerSerSerThrSerGlyIle
TCTACTGCCTTGGCCGAGCTCGCCACCAGAAGCTTTGGCAGCTCCTCACTTCCGGCATT
AGATGACGGAACCGGCTCGAGCGGTGGTCTTCGAAACCGTCGAGGAGTTGAAGGCCATA

7081 ThrGlyAspAsnThrThrThrSerSerGluProAlaProSerGlyCysProProAspSer
ACGGGCGACAATACGACAACATCTCTGAGCCCGCCCTTCTGGCTGCCCCCCGACTCC
TGCCCTCTGTTATGCTTGTAGGAGACTCGGGCGGGGAAGACCGACGGGGGGCTGAGG

(PheAla)

7141 AspAlaGluSerTyrSerSerMetProProLeuGluGlyGluProGlyAspProAspLeu
GACGCTGAGTCTATTCTCCATGCCCCCCCTGGAGGGGGAGCCTGGGGATCCGGATCTT
CTGCGACTCAGGATAAGGAGGTACGGGGGGGACCTCCCCCTCGGACCCCTAGGCCTAGAA

7201 SerAspGlySerTrpSerThrValSerSerGluAlaAsnAlaGluAspValValCysCys
AGCGACGGGTCATGGTCAACGGTCAGTAGTGAGGCCAACCGGGAGGATTCGTGTCTGCTG
TCGCTGCCAGTACCACTGTCAGTCATCACTCCGGTTGCGCTCTACGACACAGACG

7261 SerMetSerTyrSerTrpThrGlyAlaLeuValThrProCysAlaAlaGluGluGlnLys
TCAATGCTTACTCTTGACAGGCGCACTCGTCAACCCGTGCGCCGCGGAAGACAGAAA
AGTTACAGAAAGAGAACCTGTCCGCTGAGCAGTGGGGCACCGGGCGCCTTCTGCTCTT

7321 LeuProIleAsnAlaLeuSerAsnSerLeuLeuArgHisHisAsnLeuValTyrSerThr
CTGCCCATCAATGCACTAAGCAACTCGTTGTACGTACCAACAATTTGGTGTATTCCACC
GACGGGTAGTTACGTGATTGTTGAGCAACGATGCAGTGGTGTAAACCAATAGGTGG

ThrSerArgSerAlaCysGlnArgGlnLysLysValThrPheAspArgLeuGlnValLeu

Figure 1 (Sheet 8 of 10)

391 ACCTCAGCGAGTGCCTTGCCAAAGGCAGAAGAAAGTCACATTTGACAGACTGCAAGTTCTG
 TGGAGTGGTTCACGAACGGTTTCCGTCCTTTCAGTGTAAACTGTCTGACGTTCAAGAC
 AspSerHisTyrGlnAspValLeuLysGluValLysAlaAlaAlaSerLysValLysAla
 441 GACAGCCATTACCAGGACGTACTCAAGGAGGTAAAGCAGCGCGTCAAAATGAAGGC
 CTGTGGTAATGGTCTCTGCATGAGTTCCCTCCAATTCGTCCCGCAGTTTTCACITCCGA
 (Phe)
 AsnLeuLeuSerValGluGluAlaCysSerLeuThrProProHisSerAlaLysSerLys
 501 AACTTGCTATCCGTAGAGGAAGCTTGACGCTGACGCCCCACACTCAGCCAAATCCAAG
 TTGAACGATAGGCATCTCCTTCGAACGTCCGACTGCGGGGGTGTGAGTGGTTAGGTTG
 PheGlyTyrGlyAlaLysAspValArgCysHisAlaArgLysAlaValThrHisIleAsn
 561 TTGGTATGGGGCAAAAGACGTCCGTTGCCATGCCAGAAAGCCGTAAACCCACATCAAC
 AAACCAATACCCCGTTTCTGCAGGCAACGGTACGGTCTTCCGGCATTTGGGTGTAGTTG
 SerValTrpLysAspLeuLeuGluAspAsnValThrProIleAspThrThrIleMetAla
 621 TCCGTCTGGAAAGACCTTCTGGAAGACAATCTAACACCAATAGACACTACCATCATGGCT
 AGGCACACCTTCTGGAAGACCTTCTGTTACATTGTGGTTATCTGTGATGGTAGTACCGA
 LysAsnGluValPheCysValGlnProGluLysGlyGlyArgLysProAlaArgLeuIle
 681 AAGAACGAGGTTTTCTGCGTTACGCTGAGAAGGGGGTTCGTAGCCAGCTCGTCTCATC
 TTCTTGCTCCAAAGACGCAAGTCGGACTCTTCCCCCAGCATTCCGTCGAGCAGAGTAG
 ValPheProAspLeuGlyValArgValCysGluLysMetAlaLeuTyrAspValValThr
 741 GTGTTCCCGATCTGGGCGTGGCGGTGTGCGAAAGATGGCTTTGTACGACGTGGTTACA
 CACAAGGGGTAGACCCGCGACGCGCACACGCTTTCTACCGAAACATGCTGCACCAATGT
 LysLeuProLeuAlaValMetGlySerSerTyrGlyPheGlnTyrSerProGlyGlnArg
 7801 AAGCTCCCTTGGCCGTGATGGGAAGCTCCTACGGATTCCAATACTCACCAGGACAGCGG
 TCGAGGGGAACCGGCACTACCTTCGAGGATGCCTAAGGTTATGAGTGGTCTGTGCGCC
 ValGluPheLeuValGlnAlaTrpLysSerLysLysThrProMetGlyPheSerTyrAsp
 7861 GTTGAATTCCTCGTGCAAGCGTGAAGTCCAAGAAAACCCCAATGGGGTTCTCGTATGAT
 CAACTTAAGGAGCAGGTTCCGACCTTCAGGTTCTTTGGGGTTACCCCAAGAGCATACTA
 ThrArgCysPheAspSerThrValThrGluSerAspIleArgThrGluGluAlaIleTyr
 7921 ACCCGCTGCTTTGACTCCACAGTCACTGAGAGCGACATCCGTACGGAGGAGGCAATCTAC
 TGGGCGACGAACTGAGGTGTCACTGACTCTCGTGTAGGCATGCCTCCTCCGTTAGATG
 GlnCysCysAspLeuAspProGlnAlaArgValAlaIleLysSerLeuThrGluArgLeu
 7981 CAATGTTGTGACCTCGACCCCAAGCCCGGTGGCCATCAAGTCCCTCACCAGAGGCTT
 GTTACAACACTGGAGCTGGGGTTCCGGGCGCACCGGTAGTTACGGGAGTGGCTTCCGAA
 (Gly)
 TyrValGlyGlyProLeuThrAsnSerArgGlyGluAsnCysGlyTyrArgArgCysArg
 8041 TATGTTGGGGGCCCTCTTACCAATTCAAGGGGGGAGAACTGCGGCTATCGCAGGTGCCGC
 ATACAACCCCGGGAGAAATGGTTAAGTTCCCTCCTTGTACGCCGATAGCGTCCACGGCG
 AlaSerGlyValLeuThrThrSerCysGlyAsnThrLeuThrCysTyrIleLysAlaArg
 8101 GCGAGCGGCGTACTGACAACCTAGCTGTGTTAACACCCTCACTTGCTACATCAAGGCCCGG
 CGCTCGCCGATGACTGTTGATCGACACCAATTGTGGGAGTGAACGATGTAGTTCCGGGCC
 AlaAlaCysArgAlaAlaGlyLeuGlnAspCysThrMetLeuValCysGlyAspAspLeu
 8161 GCAGCCTGTGAGCCGCGAGGCTCCAGGACTGCACCATGCTCGTGTGTGGCGACGACTTA
 CGTCGGACAGCTCGGCGTCCGAGGTCTGACGTGGTACGAGCACACCCGCTGCTGAAT
 ValValIleCysGluSerAlaGlyValGlnGluAspAlaAlaSerLeuArgAlaPheThr
 8221 GTCGTTATCTGTAAAGCGCGGGGTCCAGGAGGACGCGGCGAGCCTGAGAGCCTTCAGC
 CAGCAATAGACACTTTCGCGCCCGCAGGTCTCTGCGCCGCTCGGACTCTCGGAAGTGC
 GluAlaMetThrArgTyrSerAlaProProGlyAspProProGlnProGluTyrAspLeu
 8281 GAGGCTATGACCAGGTACTCCGCCCCCCTGGGGACCCCCACACCAAGAAATACGACTTG
 CTCCGATACTGTTCCATGAGGCGGGGGGACCCCTGGGGGGTGTGGTCTTATGCTGAAC
 GluLeuIleThrSerCysSerSerAsnValSerValAlaHisAspGlyAlaGlyLysArg

Figure 1 (Sheet 9 of 10)

9341 GAGCTCATAACATCATGCTCCTCCAACGTGTCAGTCGCCCACGACGGCGCTGGAAAGAGG
 CTCGAGTATTGTAGTACGAGGAGGTTGCACASTCAGCGGGTGCTGCCGCGACCTTTCTCC
 ValTyrTyrLeuThrArgAspProThrThrProLeuAlaArgAlaAlaTrpGluThrAla
 9401 GTCTACTACCTCACCGTGACCTTACAACCCCTCGCGAGAGCTGCGTGGGAGACAGCA
 CAGATGATGGAGTGGGCACTGGGATGTTGGGGGAGCGCTCTCGACGCACCTCTGTCTG
 ArgHisThrProValAsnSerTrpLeuGlyAsnIleIleMetPheAlaProThrLeuTrp
 8461 AGACACACTCCAGTCAATTCCTGGCTAGGCAACATAATCATGTTGCCCCACACTGTGG
 TCTGTGTGAGGTCAAGTAAGGACCGATCCGTCTATTAGTACAAACGGGGGTGTGACACC
 AlaArgMetIleLeuMetThrHisPhePheSerValLeuIleAlaArgAspGlnLeuGlu
 8521 GCGAGGATGATACTGATGACCATTTCTTTAGCGTCTTATAGCCAGGGACAGCTTGAA
 CGCTCCTACTATGACTACTGGGTAAAGAAATCGCAGGAATATCGGTCCCTGGTCAACTT
 GlnAlaLeuAspCysGluIleTyrGlyAlaCysTyrSerIleGluProLeuAspLeuPro
 8581 CAGGCCCTCGATTGCGAGATCTACGGGGCCTGCTACTCCATAGAACCCTTGATCTACCT
 GTCCGGGAGCTAACGCTCTAGATGCCCCGGACGATGAGGTATCTTGGTGAAGTAGATGGA
 ProIleIleGlnArgLeuHisGlyLeuSerAlaPheSerLeuHisSerTyrSerProGly
 8641 CCAATCATTCAAAGACTCCATGGCCTCAGCGCATTTTCACTCCACAGTTACTCTCCAGGT
 GGTAGTAAGTTTCTGAGGTACCGGAGTCCGCTAAAGTGAGGTGTCAATGAGAGGTCCA
 GluIleAsnArgValAlaAlaCysLeuArgLysLeuGlyValProProLeuArgAlaTrp
 8701 GAAATTAATAGGGTGGCCGCATGCCCTCAGAAACTTGGGGTACCGCCCTTGGGAGCTTGG
 CTTTAATTATCCACCGGCGTACGGAGTCTTTTGAACCCATGGCGGGAACGCTCGAACC
 Gly
 ArgHisArgAlaArgSerValArgAlaArgLeuLeuAlaArgGlyGlyArgAlaAlaIle
 8761 AGACACCGGGCCGGAGCGTCCGCGCTAGGCTTCTGGCCAGAGGAGGCAGGGCTGCCATA
 TCTGTGGCCCCGGCCTCGCAGGCGCATCCGAAGACCGGTCTCCTCCGTCCGACGGTAT
 CysGlyLysTyrLeuPheAsnTrpAlaValArgThrLysLeuLysLeuThrProIleAla
 8821 TGTGGCAAGTACCTCTTCAACTGGGCAGTAAGAACAAGCTCAAACCTCACTCCAATAGCG
 ACACCGTTCATGGAGAAGTTGACCCGTCAATCTTGTTTTGAGTTTGAGTGAGGTTATCGC
 AlaAlaGlyGlnLeuAspLeuSerGlyTrpPheThrAlaGlyTyrSerGlyGlyAspIle
 8881 GCCGCTGGCCAGCTGGACTTGTCCGGCTGGTTACGGCTGGCTACAGCGGGGAGACATT
 CGGCGACCGGTGACCTGAACAGGCCGACCAAGTGCCGACCGATGTGCCCCCTCTGTAA
 (Pro)
 TyrHisSerValSerHisAlaArgProArgTrpIleTrpPheCysLeuLeuLeuAla
 8941 TATCACAGCGTGTCTCATGCCCGGCCCGCTGGATCTGGTTTTGCTACTCCTGCTTGCT
 ATAGTGTGCGACAGAGTACGGGCCGGGCGAATAGACCAAACGGATGAGGACGAACGA
 AlaGlyValGlyIleTyrLeuLeuProAsnArgOP
 9001 GCAGGGGTAGGCATCTACCTCCTCCCAACCGATGAAGGTTGGGGTAAACACTCCGGCCT
 CGTCCCATCCGTAGATGGAGGAGGGTTGGCTACTTCCAACCCCATTTGTGAGGCCGGA

Figure 1

Sheet 10 of 10

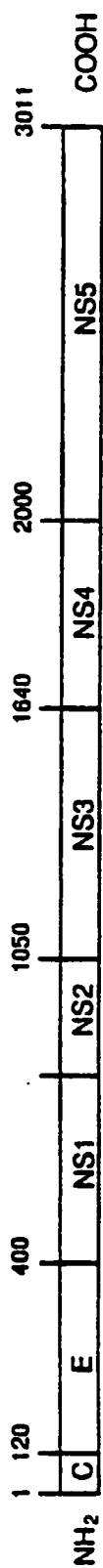


Figure 2



European Patent
Office

EUROPEAN SEARCH REPORT

Application Number

EP 91 30 2910

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
Y	WO-A-8 904 669 (CHIRON CORP.) * Page 39, lines 8-12; page 49, line 5 - page 50, line 31; page 123, line 29 - page 125, line 22; page 132, line 3 - page 134, line 35; page 171, lines 4-20 *	1-16	G 01 N 33/576 C 07 K 15/00
Y,D	EP-A-0 318 216 (CHIRON CORP.) * Page 15, line 39 - page 17, line 8; page 18, line 44 - page 19, line 13; page 27, lines 10-22 *	1-16	
A	SCIENCE, vol. 244, 21st April 1989, pages 362-364, Washington, DC, US; G. KUD et al.: "An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis" * Whole article *	1-16	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
			G 01 N C 07 K
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 02-07-1991	Examiner VAN BOHEMEN C.G.
CATEGORY OF CITED DOCUMENTS		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons A : technological background O : non-written disclosure P : intermediate document & : member of the same patent family, corresponding document	
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(54) **Combinations of hepatitis C virus (HCV) antigens for use in immunoassays for anti-HCV antibodies**

Kombinationen Hepatitis-C-Virus(HCV)-Antigene zur Anwendung in Immunoassays für Anti-HCV-Antikörper

Combinaisons d'antigènes de l'hépatitis C virus (HCV) pour usage dans des échantillons immunologiques pour anticorps anti-HCV

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- **SCIENCE**, vol. 244, 21 April 1989, Washington, DC, (US); G. KUO et al., pp. 362-364/
- **PROCEEDINGS OF THE NATL. ACADEMY OF SCIENCES USA**, vol. 89, 1992, Washington, DC (US); pp. 10011-10015/

Remarks:

The file contains technical information submitted after the application was filed and not included in this specification

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Description**Technical Field**

5 The present invention is in the field of immunoassays for HCV (previously called Non-A, Non-B hepatitis virus). More particularly, it concerns combinations of HCV antigens that permit broad range immunoassays for anti-HCV antibodies.

Background

10 The disease known previously as Non-A, Non-B hepatitis (NANBH) was considered to be a transmissible disease or family of diseases that were believed to be viral-induced, and that were distinguishable from other forms of viral-associated liver diseases, including that caused by the known hepatitis viruses, i.e., hepatitis A virus (HAV), hepatitis B virus (HBV), and delta hepatitis virus (HDV), as well as the hepatitis induced by cytomegalovirus (CMV) or Epstein-Barr virus (EBV). NANBH was first identified in transfused individuals. Transmission from man to chimpanzee and serial passage in chimpanzees provided evidence that NANBH was due to a transmissible infectious agent or agents. Epidemiologic evidence suggested that there may be three types of NANBH: a water-borne epidemic type; a blood-borne or parenterally transmitted type; and a sporadically occurring (community acquired) type. However, until recently, no transmissible agent responsible for NANBH had been identified, and clinical diagnosis and identification of NANBH had been accomplished primarily by exclusion of other viral markers. Among the methods used to detect putative NANBH antigens and antibodies were agar-gel diffusion, counterimmunoelectrophoresis, immunofluorescence microscopy, immune electron microscopy, radioimmunoassay, and enzyme-linked immunosorbent assay. However, none of these assays proved to be sufficiently sensitive, specific, and reproducible to be used as a diagnostic test for NANBH.

15 In 1987, scientists at Chiron Corporation (the owner of the present application) identified the first nucleic acid definitively linked to blood-borne NANBH. See, e.g., EPO Pub. No. 318,216: Houghton *et al.*, Science **244**:359 (1989). These publications describe the cloning of an isolate from a new viral class, hepatitis C virus (HCV), the prototype isolate described therein being named "HCV1". HCV is a Flavi-like virus, with an RNA genome.

20 US Patent 5,350,671 (Houghton *et al.*), incorporated herein by reference, describes the preparation of various recombinant HCV polypeptides by expressing HCV cDNA and the screening of those polypeptides for immunological reactivity with sera from HCV patients. That limited screening showed that at least five of the polypeptides tested were very immunogenic; specifically, those identified as 5-1-1, C100, C33c, CA279a, and CA290a. Of these five polypeptides, 5-1-1 is located in the putative NS4 domain; C100 spans the putative NS3 and NS4 domains; C33c is located within the putative NS3 domain and CA279a and CA290a are located within the putative C domain. The screening also showed that no single polypeptide tested was immunologically reactive with all sera. Thus, improved tests, which react with all or more samples from HCV positive individuals, are desirable.

25 EP-A-445,423, filed on 22nd December 1990 and published on 11th September 1991 describes immunoassays for HCV. EP-A-445,423 describes the use of the C100-3 recombinant yeast/hepatitis C virus SOD fusion polypeptide (disclosed in EP-A-318,216) together with a polypeptide selected from the group consisting of, *inter alia*, p1, p35 and p99. The peptide p1 corresponds to amino acids residues 1 to 75 of Figure 1A (where position 9 is Lys and 11 is Asn), p35 corresponds to amino acid residues 35 to 75 of Figure 1A, and p99 corresponds to residues 99 to 126 of Figure 1A.

30 WO91/15574, published on 17 October 1991 describes, *inter alia*, purified proteins and glycopeptides of HCV useful in a diagnostic system for detection of human HCV antisera. EP-A-442 394 describes synthetic peptides for the detection of antibodies to HCV.

Disclosure of the Invention

45 Applicants have carried out additional serological studies on HCV antigens that confirm that no single HCV polypeptide identified to date is immunologically reactive with all sera. This lack of a single polypeptide that is universally reactive with all sera from individuals with HCV may be due, *inter alia*, to strain-to-strain variation in HCV epitopes, variability in the humoral response from individual-to-individual and/or variation in serology with the state of the disease.

50 These additional studies have also enabled applicants to identify combinations of HCV antigens that provide more efficient detection of HCV antibodies than any single HCV polypeptide.

Accordingly, one aspect of this invention is a combination of hepatitis C virus (HCV) epitope sequences in one or more polypeptides made by chemical synthesis or recombinant expression, immobilized on the surface of a solid matrix suitable for detection of HCV by immunoassay, comprising:

- (a) a first epitope sequence from the C domain of the HCV polypeptide;
- (b) a second epitope sequence from a second domain of the HCV polypeptide which domain is:

- (i) the NS3 domain of the HCV polyprotein;
- (ii) the NS4 domain of the HCV polyprotein; or
- (iii) the NS5 domain of the HCV polyprotein;

5 and

(c) a third epitope sequence from a third domain of the HCV polyprotein which domain is:

- (i) the NS3 domain of the HCV polyprotein;
- (ii) the NS4 domain of the HCV polyprotein; or
- (iii) the NS5 domain of the HCV polyprotein;

wherein the third domain is different from the second domain; with the proviso that the combination is not the peptide pl with C100-3, the peptide p35 with C100-3 or the peptide p99 with C100-3.

15 In one embodiment, the combination of HCV epitope sequences is in the form of a fusion protein comprised of the epitopes. In an alternative embodiment, the combination of epitope sequences is in the form of the individual epitopes bound to a common solid matrix. In still another embodiment, the combination of epitope sequences is in the form of a mixture of the individual epitopes.

20 Another aspect of the invention is a method for detecting antibodies to HCV in a mammalian body component suspected of containing said antibodies comprising contacting said body component with the above-described combination of HCV epitope sequences under conditions that permit antibody-antigen reaction and detecting the presence of immune complexes of said antibodies and said epitope sequences.

Said body component may be contacted with a panel of HCV epitope sequences simultaneously or sequentially.

25 Another aspect of the invention is a kit for carrying out an assay for detecting antibodies to HCV in a mammalian body component suspected of containing said antibodies comprising in packaged combination

- (a) said combination of HCV epitope sequences;
- (b) standard control reagents; and
- (c) instructions for carrying out the assay.

30

Brief Description of the Drawings

In the drawings:

35 Figure 1 is the nucleotide sequence of the cDNA sense and anti-sense strand for the HCV polyprotein and the amino acid sequence encoded by the sense strand.

Figure 2 is a schematic of the amino acid sequence of Figure 1 showing the putative domains of the HCV polypeptide.

Modes for Carrying Out the Invention

40

Definitions

"HCV antigen" means a polypeptide of at least about 5 amino acids, more usually at least about 8 to 10 amino acids that defines an epitope found in an isolate of HCV. Preferably, the epitope is unique to HCV. When an antigen is 45 designated by an alphanumeric code, the epitope is from the HCV domain specified by the alphanumeric.

"Synthetic" as used to characterize an HCV antigen means that the HCV antigen has been man-made such as by chemical or recombinant synthesis.

50 "Domains" means those segments of the HCV polyprotein shown in Figure 2 which generally correspond to the putative structural and nonstructural proteins of HCV. Domain designations generally follow the convention used to name Flaviviral proteins. The locations of the domains shown in Figure 2 are only approximate. The designations "NS" denotes "nonstructural" domains, while "S" denotes the envelope domain, and "C" denotes the nucleocapsid or core domain.

"Fusion polypeptide" means a polypeptide in which the HCV antigen(s) are part of a single continuous chain of amino acids, which chain does not occur in nature. The HCV antigens may be connected directly to each other by peptide bonds or be separated by intervening amino acid sequences. The fusion polypeptides may also contain amino acid sequences exogenous to HCV.

55 "Common solid matrix" means a solid body to which the individual HCV antigens or the fusion polypeptide comprised of HCV antigens are bound covalently or by noncovalent means such as hydrophobic adsorption.

"Mammalian body component" means a fluid or tissue of a mammalian individual (e.g., a human) that commonly contains antibodies produced by the individual. Such components are known in the art and include, without limitation, blood, plasma, serum, spinal fluid, lymph fluid, secretions of the respiratory, intestinal or genitourinary tracts, tears, saliva, milk, white blood cells, and myelomas.

"Immunologically reactive" means that the antigen in question will react specifically with anti-HCV antibody commonly present in a significant proportion of sera from individuals infected with HCV.

"Immune complex" means the combination or aggregate formed when an antibody binds to an epitope on an antigen.

10 Combinations of HCV Antigens

Figure 2 shows the putative domains of the HCV polyprotein. The domains from which the antigens used in the combinations derive are: C, S (or E), NS3, NS4, and NS5. The C domain is believed to define the nucleocapsid protein of HCV. It extends from the N-terminal of the polyprotein to approximately amino acid 120 of Figure 1. The S domain is believed to define the virion envelope protein, and possibly the matrix (M) protein, and is believed to extend from approximately amino acid 120 to amino acid 400 of Figure 1. The NS3 domain extends from approximately amino acid 1050 to amino acid 1640 and is believed to constitute the viral protease. The NS4 domain extends from the terminus of NS3 to approximately amino acid 2000. The function of the NS4 protein is not known at this time. Finally, the NS5 domain extends from about amino acid 2000 to the end of the polyprotein and is believed to define the viral polymerase.

The sequence shown in Figure 1 is the sequence of the HCV1 isolate. It is expected that the sequences of other strains of the blood-borne HCV may differ from the sequence of Figure 1, particularly in the envelope (S) and nucleocapsid (C) domains. The use of HCV antigens having such differing sequences is intended to be within the scope of the present invention, provided, however, that the variation does not significantly degrade the immunological reactivity of the antigen to sera from persons infected with HCV.

In general, the HCV antigens will comprise entire or truncated domains, the domain fragments being readily screened for antigenicity by those skilled in the art. The individual HCV antigens used in the combination will preferably comprise the immunodominant portion (i.e., the portion primarily responsible for the immunological reactivity of the polypeptide) of the stated domain. In the case of the C domain it is preferred that the C domain antigen comprise a majority of the entire sequence of the domain. The antigen designated C22 (see Example 4, *infra*), is particularly preferred. The S domain antigen preferably includes the hydrophobic subdomain at the N-terminal end of the domain. This hydrophobic subdomain extends from approximately amino acid 199 to amino acid 328 of Figure 1. The HCV antigen designated S2 (see Example 3, *infra*), is particularly preferred. Sequence downstream of the hydrophobic subdomain may be included in the S domain antigen if desired.

A preferred NS3 domain antigen is the antigen designated C33c. That antigen includes amino acids 1192 to 1457 of Figure 1. A preferred NS4 antigen is C100 which comprises amino acids 1569 to 1931 of Figure 1. A preferred NS5 antigen comprises amino acids 2054 to 2464 of Figure 1.

The HCV antigen may be in the form of a polypeptide composed entirely of HCV amino acid sequence or it may contain sequence exogenous to HCV (i.e., it may be in the form of a fusion protein that includes exogenous sequence). In the case of recombinantly produced HCV antigen, producing the antigen as a fusion protein such as with SOD, alpha-factor or ubiquitin (see commonly owned U.S. Pat. No. 4,751,180, U.S. Pat. No. 4,870,008 and U.S. Pat. Application Serial. No. 390,599, filed 7 August 1989, which describe expression of SOD, alpha-factor and ubiquitin fusion proteins) may increase the level of expression and/or increase the water solubility of the antigen. Fusion proteins such as the alpha-factor and ubiquitin fusion are processed by the expression host to remove the heterologous sequence. Alpha-factor is a secretion system, however, while ubiquitin fusions remain in the cytoplasm.

Further, the combination of antigens may be produced as a fusion protein. For instance, a continuous fragment of DNA encoding C22 and C33c may be constructed, cloned into an expression vector and used to express a fusion protein of C22 and C33c. In a similar manner fusion proteins of C22 and C100; C22 and S2; C22 and an NS5 antigen; C22, C33c, and S2; C22, C100 and S2, and C22, C33c, C100, and S2 may be made. Alternative fragments from the exemplified domain may also be used.

50 Preparation of HCV Antigens

The HCV antigens of the invention are preferably produced recombinantly or by known solid phase chemical synthesis. They may, however, also be isolated from dissociated HCV or HCV particles using affinity chromatography techniques employing antibodies to the antigens.

When produced by recombinant techniques, standard procedures for constructing DNA encoding the antigen, cloning that DNA into expression vectors, transforming host cells such as bacteria, yeast, insect, or mammalian cells, and expressing such DNA to produce the antigen may be employed. As indicated previously, it may be desirable to

express the antigen as a fusion protein to enhance expression, facilitate purification, or enhance solubility. Examples of specific procedures for producing representative HCV antigens are described in the Examples, infra, and in US patent 5,350,671.

5 Formulation of Antigens for Use in Immunoassay

The HCV antigens may be combined by producing them in the form of a fusion protein composed of two or more of the antigens, by immobilizing them individually on a common solid matrix, or by physically mixing them. Fusion proteins of the antigen may also be immobilized on (bound to) a solid matrix. Methods and means for covalently or noncovalently binding proteins to solid matrices are known in the art. The nature of the solid surface will vary depending upon the assay format. For assays carried out in microtiter wells, the solid surface will be the wall of the well or cup. For assays using beads, the solid surface will be the surface of the bead. In assays using a dipstick (i.e., a solid body made from a porous or fibrous material such as fabric or paper) the surface will be the surface of the material from which the dipstick is made. In agglutination assays the solid surface may be the surface of latex or gelatin particles. When individual antigens are bound to the matrix they may be distributed homogeneously on the surface or distributed thereon in a pattern, such as bands so that a pattern of antigen binding may be discerned.

Simple mixtures of the antigens comprise the antigens in any suitable solvent or dispersing medium.

20 Assay Formats Using Combinations of Antigens

The HCV antigens may be employed in virtually any assay format that employs a known antigen to detect antibodies. A common feature of all of these assays is that the antigen is contacted with the body component suspected of containing HCV antibodies under conditions that permit the antigen to bind to any such antibody present in the component. Such conditions will typically be physiologic temperature, pH and ionic strength using an excess of antigen. The incubation of the antigen with the specimen is followed by detection of immune complexes comprised of the antigen.

Design of the immunoassays is subject to a great deal of variation, and many formats are known in the art. Protocols may, for example, use solid supports, or immunoprecipitation. Most assays involve the use of labeled antibody or polypeptide; the labels may be, for example, enzymatic, fluorescent, chemiluminescent, radio-active, or dye molecules. Assays which amplify the signals from the immune complex are also known; examples of which are assays which utilize biotin and avidin, and enzyme-labeled and mediated immunoassays, such as ELISA assays.

The immunoassay may be, without limitation, in a heterogeneous or in a homogeneous format, and of a standard or competitive type. In a heterogeneous format, the polypeptide is typically bound to a solid matrix or support to facilitate separation of the sample from the polypeptide after incubation. Examples of solid supports that can be used are nitro-cellulose (e.g., in membrane or microtiter well form), polyvinyl chloride (e.g., in sheets or microtiter wells), polystyrene latex (e.g., in beads or microtiter plates, polyvinylidene fluoride (known as Immulon™), diazotized paper, nylon membranes, activated beads, and Protein A beads. For example, Dynatech Immulon™ 1 or Immulon™ 2 microtiter plates or 6.4 mm (0.25 inch) polystyrene beads (Precision Plastic Ball) can be used in the heterogeneous format. The solid support containing the antigenic polypeptides is typically washed after separating it from the test sample, and prior to detection of bound antibodies. Both standard and competitive formats are known in the art.

In a homogeneous format, the test sample is incubated with the combination of antigens in solution. For example, it may be under conditions that will precipitate any antigen-antibody complexes which are formed. Both standard and competitive formats for these assays are known in the art.

In a standard format, the amount of HCV antibodies forming the antibody-antigen complex is directly monitored. This may be accomplished by determining whether labeled anti-xenogenic (e.g., anti-human) antibodies which recognize an epitope on anti-HCV antibodies will bind due to complex formation. In a competitive format, the amount of HCV antibodies in the sample is deduced by monitoring the competitive effect on the binding of a known amount of labeled antibody (or other competing ligand) in the complex.

Complexes formed comprising anti-HCV antibody (or, in the case of competitive assays, the amount of competing antibody) are detected by any of a number of known techniques, depending on the format. For example, unlabeled HCV antibodies in the complex may be detected using a conjugate of anti-xenogenic Ig complexed with a label, (e.g., an enzyme label).

In an immunoprecipitation or agglutination assay format the reaction between the HCV antigens and the antibody forms a network that precipitates from the solution or suspension and forms a visible layer or film of precipitate. If no anti-HCV antibody is present in the test specimen, no visible precipitate is formed.

The HCV antigens will typically be packaged in the form of a kit for use in these immunoassays. The kit will normally contain in separate containers the combination of antigens (either already bound to a solid matrix or separate with reagents for binding them to the matrix), control antibody formulations (positive and/or negative), labeled antibody when the assay format requires same and signal generating reagents (e.g., enzyme substrate) if the label does not

generate a signal directly. Instructions (e.g., written, tape, VCR, CD-ROM, etc.) for carrying out the assay usually will be included in the kit.

The following examples are intended to illustrate the invention and are not intended to limit the invention in any manner.

Example 1: Synthesis of HCV Antigen C33c

HCV antigen C33c contains a sequence from the NS3 domain. Specifically, it includes amino acids 1192-1457 of Figure 1. This antigen was produced in bacteria as a fusion protein with human superoxide dismutase (SOD) as follows. The vector pSODcfl (Steiner et al. (1986), J. Virol. 58:9) was digested to completion with EcoRI and BamHI and the resulting EcoRI, BamHI fragment was ligated to the following linker to form pcf1EF:

GATC CTG GAA TTC TGA TAA
GAC CTT AAG ACT ATT TTA A

A cDNA clone encoding amino acids 1192-1457 and having EcoRI ends was inserted into pcf1EF to form pcf1EF/C33c. This expression construct was transformed into D1210 *E. coli* cells.

The transformants were used to express a fusion protein comprised of SOD at the N-terminus and in-frame C33c HCV antigen at the C-terminus. Expression was accomplished by inoculating 1500 ml of Luria broth containing ampicillin (100 micrograms/ml) with 15 ml of an overnight culture of the transformants. The cells were grown to an O.D. of 0.3, IPTG was added to yield a final concentration of 2 mM, and growth continued until the cells attained a density of 1 O.D., at which time they were harvested by centrifugation at 3,000 x g at 4°C for 20 minutes. The packed cells can be stored at -80°C for several months.

In order to purify the SOD-C33c polypeptide the bacterial cells in which the polypeptide was expressed were subjected to osmotic shock and mechanical disruption, the insoluble fraction containing SOD-C33c was isolated and subjected to differential extraction with an alkaline-NaCl solution, and the fusion polypeptide in the extract purified by chromatography on columns of S-Sepharose(TM) and Q-sepharose(TM).

The crude extract resulting from osmotic shock and mechanical disruption was prepared by the following procedure. One gram of the packed cells were suspended in 10 ml of a solution containing 0.02 M Tris HCl, pH 7.5, 10 mM EDTA, 20% sucrose, and incubated for 10 minutes on ice. The cells were then pelleted by centrifugation at 4,000 x g for 15 min at 4°C. After the supernatant was removed, the cell pellets were resuspended in 10 ml of Buffer A1 (0.01M Tris HCl, pH 7.5, 1 mM EDTA, 14 mM betamercaptoethanol [BME]), and incubated on ice for 10 minutes. The cells were again pelleted at 4,000 x g for 15 minutes at 4°C. After removal of the clear supernatant (periplasmic fraction I), the cell pellets were resuspended in Buffer A1, incubated on ice for 10 minutes, and again centrifuged at 4,000 x g for 15 minutes at 4°C. The clear supernatant (periplasmic fraction II) was removed, and the cell pellet resuspended in 5 ml of Buffer A2 (0.02 M Tris HCl, pH 7.5, 14 mM BME, 1 mM EDTA, 1 mM PMSF). In order to disrupt the cells, the suspension (5 ml) and 7.5 ml of Dyno-mill lead-free acid washed glass beads (0.10-0.15 mm diameter)(obtained from Glen-Mills, Inc.) were placed in a Falcon tube, and vortexed at top speed for two minutes, followed by cooling for at least 2 min on ice; the vortexing-cooling procedure was repeated another four times. After vortexing, the slurry was filtered through a scintered glass funnel using low suction; the glass beads were washed two times with Buffer A2, and the filtrate and washes combined.

The insoluble fraction of the crude extract was collected by centrifugation at 20,000 x g for 15 min at 4°C, washed twice with 10 ml Buffer A2, and resuspended in 5 ml of MILLI-Q(TM)water.

A fraction containing SOD-C33c was isolated from the insoluble material by adding to the suspension NaOH (2 M) and NaCl (2 M) to yield a final concentration of 20 mM each, vortexing the mixture for 1 minute, centrifuging it 20,000 x g for 20 min at 4°C, and retaining the supernatant.

In order to purify SOD-C33c on S-Sepharose(TM), the supernatant fraction was adjusted to a final concentration of 6M urea, 0.05M Tris HCl, pH 7.5, 14 mM BME, 1 mM EDTA. This fraction was then applied to a column of S-Sepharose(TM) Fast Flow (1.5 x 10 cm) which had been equilibrated with Buffer B (0.05M Tris HCl, pH 7.5, 14 mM BME, 1 mM EDTA). After application, the column was washed with two column volumes of Buffer B. The flow through and wash fractions were collected. The flow rate of application and wash, was 1 ml/min; and collected fractions were 1 ml. In order to identify fractions containing SOD-C33c, aliquots of the fractions were analyzed by electrophoresis on 10% polyacrylamide gels containing SDS followed by staining with Coomassie blue. The fractions are also analyzable by Western blots using an antibody directed against SOD. Fractions containing SOD-C33c were pooled.

Further purification of SOD-C33c was on a Q-Sepharose(TM) column (1.5 x 5 cm) which was equilibrated with Buffer B. The pooled fractions containing SOD-C33c obtained from chromatography on S-Sepharose(TM) was applied to the column. The column was then washed with Buffer B, and eluted with 60 ml of a gradient of 0.0 to 0.4 M NaCl in Buffer B. The flow rate for application, wash, and elution was 1 ml/min; collected fractions were 1 ml. All fractions from

the Q-Sepharose(TM) column were analyzed as described for the S-Sepharose(TM) column. The peak of SOD-C33c eluted from the column at about 0.2 M NaCl.

The SOD-C33c obtained from the Q-Sepharose(TM) column was greater than about 90% pure, as judged by analysis on the polyacrylamide SDS gels and immunoblot using a monoclonal antibody directed against human SOD.

Example 2: Synthesis of HCV Antigen C100

HCV antigen C100 contains sequences from the NS3 and NS4 domains. Specifically, it includes amino acids 1569-1931 of Figure 1. This antigen was produced in yeast. A cDNA fragment of a 1270 bp encoding the above amino acids and heaving EcoRI termini was prepared.

The construction of a yeast expression vector in which this fragment was fused directly to the *S. cerevisiae* ADH2/GAP promoter was accomplished by a protocol which included amplification of the C100 sequence using a PCR method, followed by ligation of the amplified sequence into a cloning vector. After cloning, the C100 sequence was excised, and with a sequence which contained the ADH2/GAP promoter, was ligated to a large fragment of a yeast vector to yield a yeast expression vector.

The PCR amplification of C100 was performed using as template the vector pS3-56_{C100m}, which had been linearized by digestion with Sall. pS3-56, which is a pBR322 derivative, contains an expression cassette which is comprised of the ADH2/GAPDH hybrid yeast promoter upstream of the human superoxide dismutase gene, and a downstream alpha factor transcription terminator.

The oligonucleotide primers used for the amplification were designed to facilitate cloning into the expression vector, and to introduce a translation termination codon. Specifically, novel 5'-HindIII and 3'-Sall sites were generated with the PCR oligonucleotides. The oligonucleotide containing the Sall site also encodes the double termination codons, TAA and TGA. The oligonucleotide containing the HindIII site also contains an untranslated leader sequence derived from the pgap63 gene, situated immediately upstream of the AUG codon. The pEco63GAPDH gene is described by Holland and Holland (1980) and by Kniskern et al. (1986). The PCR primer sequences used for the direct expression of C100m were:

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5' GAG TGC TCA AGC TTC AAA ACA AAA TGG CTC
   ACT TTC TAT CCC AGA CAA AGC AGA GT 3'
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and

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5' GAG TGC TCG TCG ACT CAT TAG GGG GAA
   ACA TGG TTC CCC CGG GAG GCG AA 3'.
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Amplification by PCR, utilizing the primers, and template, was with a Cetus-Perkin-Elmer PCR kit, and was performed according to the manufacturer's directions. The PCR conditions were 29 cycles of 94°C for a minute, 37°C for 2 minutes, 72°C for 3 minutes; and the final incubation was at 72°C for 10 minutes. The DNA can be stored at 4°C or -20°C overnight.

After amplification, the PCR products were digested with HindIII and Sall. The major product of 1.1 kb was purified by electrophoresis on a gel, and the eluted purified product was ligated with a large Sall-HindIII fragment of pBR322. In order to isolate correct recombinants, competent HB101 cells were transformed with the recombinant vectors, and after cloning, the desired recombinants were identified on the basis of the predicted size of HindIII-Sall fragments excised from the clones. One of the clones which contained the a HindIII-Sall fragment of the correct size was named pBR322/C100-d. Confirmation that this clone contained amplified C100 was by direct sequence analysis of the HindIII-Sall fragment.

The expression vector containing C100 was constructed by ligating the HindIII-Sall fragment from pBR322/C100-d to a 13.1 kb BamHI-Sall fragment of pBS24.1, and a 1369 bp BamHI-HindIII fragment containing the ADH2/GAP promoter. (The latter fragment is described in EPO 164,556). The ADH2/GAP promoter fragment was obtained by digestion of the vector pPGAP/AG/HindIII with HindIII and BamHI, followed by purification of the 1369 bp fragment on a gel.

Competent HB101 cells were transformed with the recombinant vectors; and correct recombinants were identified by the generation of a 2464 bp fragment and a 13.1 kb fragment generated by BamHI and Sall digestion of the cloned vectors. One of the cloned correct recombinant vectors was named pC100-d#3.

In order to express C100, competent cells of *Saccharomyces cerevisiae* strain AB122 (MATa leu2 ura3-53 prb 1-1122 pep4-3 prc1-407[cir-0]) were transformed with the expression vector pC100-d#3. The transformed cells were plated on URA-sorbitol, and individual transformants were then streaked on Leu^r plates.

Individual clones were cultured in Leu^r, ura^r medium with 2% glucose at 30°C for 24-36 hours. One liter of Yeast

Extract Peptone Medium (YEP) containing 2% glucose was inoculated with 10 ml of the overnight culture, and the resulting culture was grown at 30°C at an agitation rate of 400 rpm and an aeration rate of 1 L of air per 1 L of medium per minute (i.e., 1vvm) for 48 hours. The pH of the medium was not controlled. The culture was grown in a BioFlo II fermentor manufactured by New Brunswick Science Corp. Following fermentation, the cells were isolated and analyzed for C100 expression.

Analysis for expressed C100 polypeptide by the transformed cells was performed on total cell lysates and crude extracts prepared from single yeast colonies obtained from the Leu^r plates. The cell lysates and crude extracts were analyzed by electrophoresis on SDS polyacrylamide gels, and by Western blots. The Western blots were probed with rabbit polyclonal antibodies directed against the SOD-C100 polypeptide expressed in yeast. The expected size of the C100 polypeptide is 364 amino acids. By gel analysis the expressed polypeptide has a MW_r of 39.9K.

Both analytical methods demonstrated that the expressed C100 polypeptide was present in total cell lysates, but was absent from crude extracts. These results suggest that the expressed C100 polypeptide may be insoluble.

Example 3: Expression of HCV Antigen S2

HCV antigen S2 contains a sequence from the hydrophobic N-terminus of the S domain. It includes amino acids 199-328 of Figure 1.

The protocol for the construction of the expression vector encoding the S2 polypeptide and for its expression in yeast was analogous to that used for the expression of the C100 polypeptide, described in Example 2.

The template for the PCR reaction was the vector pBR322/Pi14a, which had been linearized by digestion with HindIII. Pi14a is a cDNA clone that encodes amino acids 199-328.

The oligonucleotides used as primers for the amplification by PCR of the S2 encoding sequence were the following. For the 5'-region of the S2 sequence:

5' GAG TGC TCA AGC TTC AAA ACA AAA TGG GGC TCT
ACC ACG TCA CCA ATG ATT GCC CTA AC 3';

and

for the 3'-region of the S2 sequence:

5' GAG TGC TCG TCG ACT CAT TAA GGG GAC CAG TTC
ATC ATC ATA TCC CAT GCC AT 3'.

The primer for the 5'-region introduces a HindIII site and an ATG start codon into the amplified product. The primer for the 3'-region introduces translation stop codons and a Sall site into the amplified product.

The PCR conditions were 29 cycles of 94°C for a minute, 37°C for 2 minutes, 72°C for 3 minutes, and the final incubation was at 72°C for 10 minutes.

The main product of the PCR reaction was a 413 bp fragment, which was gel purified. The purified fragment was ligated to the large fragment obtained from pBR322 digested with HindIII and Sall fragment, yielding the plasmid pBR322/S2d.

Ligation of the 413 bp HindIII-Sall S2 fragment with the 1.36 kb BamHI-HindIII fragment containing the ADH2/GAP promoter, and with the large BamHI-Sall fragment of the yeast vector pBS24.1 yielded recombinant vectors, which were cloned. Correct recombinant vectors were identified by the presence of a 1.77 kb fragment after digestion with BamHI and Sall. An expression vector constructed from the amplified sequence, and containing the sequence encoding S2 fused directly to the ADH2/GAP promoter is identified as pS2d#9.

Example 4: Synthesis of HCV C Antigen

HCV antigen C22 is from the C domain. It comprises amino acids 1-122 of Figure 1.

The protocol for the construction of the expression vector encoding the C polypeptide and for its expression in yeast was analogous to that used for the expression of the C100 polypeptide, described supra, except for the following.

The template for the PCR reaction was pBR322/ Ag30a which had been linearized with HindIII. Ag30 is a cDNA clone that encodes amino acids 1-122. The oligonucleotides used as primers for the amplification by PCR of the C encoding sequence were the following.

For the 5'-region of the C sequence:

5' GAG TGC AGC TTC AAA ACA AAA TGA GCA CGA
ATC CTA AAC CTC AAA AAA AAA AC 3',

5 and
for the 3'-region of the C sequence:

5' GAG TGC TCG TCG ACT CAT TAA CCC AAA TTG CGC
GAC CTA CGC CGG GGG TCT GT 3'.

The primer for the 5'-region introduces a HindIII site into the amplified product, and the primer for the 3'-region introduces translation stop codons and a Sall site. The PCR was run for 29 cycles of 94°C for a minute, 37°C for 2 minutes, 72°C for 3 minutes, and the final incubation was at 72°C for 10 minutes.

The major product of PCR amplification is a 381 bp polynucleotide. Ligation of this fragment with the Sall-HindIII large Sall-HindIII fragment of pBR322 yielded the plasmid pBR322/C2.

Ligation of the 381 bp HindIII-Sall C coding fragment excised from pBR322/C2 with the 1.36 kb BamHI-HindIII fragment containing the ADH2/GAP promoter, and with the large BamHI-Sall fragment of the yeast vector pBS24.1 yielded recombinant vectors, which were cloned. Correct recombinant vectors were identified by the presence of a 1.74 kb fragment after digestion with BamHI and Sall. An expression vector constructed from the amplified sequence, and containing the sequence encoding C fused directly to the ADH2/GAP promoter is identified as pC22.

Analysis for expressed C polypeptide by the transformed cells was performed on total cell lysates and crude extracts prepared from single yeast colonies obtained from the Leu⁻ plates. The cell lysates and crude extracts were analyzed by electrophoresis on SDS polyacrylamide gels. The C polypeptide is expected to have 122 amino acids and by gel analysis the expressed polypeptide has a MW_r of approximately 13.6 Kd.

Example 5: Synthesis of NS5 Polypeptide

This polypeptide contains sequence from the N-terminus of the NS5 domain. Specifically it includes amino acids 2054 to 2464 of Figure 1. The protocol for the construction of the expression vector encoding the NS5 polypeptide and for its expression were analogous to that used for the expression of C33c (see Example 1).

Example 6: Radioimmunoassay (RIA) for Antibodies to HCV

The HCV antigens of Examples 1-5 were tested in an RIA format for their ability to detect antibodies to HCV in the serum of individuals clinically diagnosed as having HCV (Non-A, Non-B) and in serum from blood given by paid blood donors.

The RIA was based upon the procedure of Tsu and Herzenberg (1980) in SELECTED METHODS IN CELLULAR IMMUNOLOGY (W.H. Freeman & Co.), pp. 373-391. Generally, microtiter plates (Immulon 2, Removawell strips) are coated with purified HCV antigen. The coated plates are incubated with the serum samples or appropriate controls. During incubation, antibody, if present, is immunologically bound to the solid phase antigen. After removal of the unbound material and washing of the microtiter plates, complexes of human antibody-NANBV antigen are detected by incubation with ¹²⁵I-labeled sheep anti-human immunoglobulin. Unbound labeled antibody is removed by aspiration, and the plates are washed. The radioactivity in individual wells is determined; the amount of bound human anti-HCV antibody is proportional to the radioactivity in the well.

Specifically, one hundred microliter aliquots containing 0.1 to 0.5 micrograms of the HCV antigen in 0.125 M Na borate buffer, pH 8.3, 0.075 M NaCl (BBS) was added to each well of a microtiter plate (Dynatech Immulon 2 Removawell Strips). The plate was incubated at 4°C overnight in a humid chamber, after which, the antigen solution was removed and the wells washed 3 times with BBS containing 0.02% Triton X-100 (TM) (BBST). To prevent non-specific binding, the wells were coated with bovine serum albumin (BSA) by addition of 100 microliters of a 5 mg/ml solution of BSA in BBS followed by incubation at room temperature for 1 hour; after this incubation the BSA solution was removed. The antigens in the coated wells were reacted with serum by adding 100 microliters of serum samples diluted 1:100 in 0.01M Na phosphate buffer, pH 7.2, 0.15 M NaCl (PBS) containing 10 mg/ml BSA, and incubating the serum containing wells for 1 hr at 37°C. After incubation, the serum samples were removed by aspiration, and the wells were washed 5 times with BBST. Antibody bound to the antigen was determined by the binding of ¹²⁵I-labeled F'(ab)₂ sheep anti-human IgG to the coated wells. Aliquots of 100 microliters of the labeled probe (specific activity 5-20 microcuries/microgram) were added to each well, and the plates were incubated at 37°C for 1 hour, followed by removal of excess probe by aspiration, and 5 washes with BBST. The amount of radioactivity bound in each well was determined by counting in a counter which detects gamma radiation.

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Table 1 below presents the results of the tests on the serum from individuals diagnosed as having HCV.

Table 1

	<u>INDIVIDUAL</u>	<u>ANTIGEN</u>				
		<u>S2</u>	<u>C22</u>	<u>C100</u>	<u>C33c</u>	<u>NS5</u>
5						
	CVH IVDA	P	P	P(+++)	P	P
10	CVH IVDA	P	P	P(+)	P	P
	CVH IVDA	P	P	P(+)	P	P
	CVH NOS	P	P	P	P	P
15	AVH NOS HS	N	N	N	N	N
	AVH NOS HS	P	N	N	N	N
	AVH NOS HS	P	N	N	N	N
	AVH NOS HS	P/N	N	N	N	N
20	AVH PTVH	N	N	N	P/N	N
	AVH NOS HS	N	N	N	N	N
	AVH NOS	N	N	N	N	P
25	AVH PTVH	N	N	N	N	N
	AVH IVDA	N	P	N	P	P
	AVH PTVH	P	P/N	N	N	P
	AVH NOS	N	P	N	N	N
30	AVH IVDA	N	P	N	P	P
	AVH NOS HS	P/N	N	N	N	N
	AVH PTVH	N	N	N	N	N
35	CVH IVDA	P	P	P	P	P
	CVH IVDA	P	P	P	P	P
	AVH NOS HS	N	N	N	N	N
40	CVH PTVH	P	P	N	N	N
	AVH PTVH	P	N	P(+)	P(+++)	N
	CVH PTVH	N	P	P	P	P
	CVH NOS HS	P	P	P	P	N
45	CVH NOS	N	P	P/N	P	P
50						
55						

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<u>INDIVIDUAL</u>		<u>ANTIGEN</u>				
		<u>S2</u>	<u>C22</u>	<u>C100</u>	<u>C33c</u>	<u>NS5</u>
5	CVH IVDA	N	N	N	P	N
	AVH IVDA	P	P	P	P	P
	AVH IVDA	P	P	P	P	P
	CVH IVDA	P	P	P	P	P
10	AVH IVDA	P/N	P	N	P	P
	AVH IVDA	N	P	P	P	N
	CVH PTVH	P	P/N	N	N	N
15	CVH NOS	N	N	N	N	N
	CVH NOS	N	N	N	N	N
	CVH IVDA	P	P	P	P	P
20	AVH IVDA	P	P	P	P	P
	CVH PTVH	P	P	P	P	P
	AVH PTVH?	N	P	P	P	P
	AVH IVDA	N	P	N	P	N
25	AVH NOS	N	N	N	N	N
	AVH NOS	N	N	N	N	N
	CVH NOS	N	P	N	N	P
30	CVH NOS	P	P	N	N	N
	CVH NOS HS	P	P	P	P	P
	CVH PTVH	P	P	N	P	P
	AVH nurse	P	P	N	N	N
35	AVH IVDA	P	P	P	P	N
	AVH IVDA	N	P	P(+)	P(+++)	N
	AVH nurse	P/N	P	N	N	N
40	AVH PTVH	P/N	P	P	N	P
	AVH NOS	N	P/N	N	N	P
	AVH NOS	N	P	N	P	N
	AVH PTVH	P	P/N	N	N	N
45	AVH PTVH	N	P	N	P	P
	AVH PTVH	P	P	P	P	P
	AVH PTVH	N	P	N	N	P
50	CVH PTVH	P/N	P	P(+)	P(+++)	N
	AVH PTVH	N	P/N	P(+)	P(+++)	P

55

<u>INDIVIDUAL</u>		<u>ANTIGEN</u>			
		<u>S2</u>	<u>C22</u>	<u>C100</u>	<u>C33c</u> <u>NS5</u>
5	AVH PTVH	P	(?)	P	N P
	CVH PTVH	N	P	N	P P
	CVH PTVH	N	P	P	P P
	CVH PTVH	N	N	N	N N
10	AVH NOS	N	N	N	N N
	AVH nurse	P	P	N	N N
	CVH PTVH	N	P	N	N P
15	AVH IVDA	N	P	N	P/N N
	CVH PTVH	P	P	P(+)	P(+++) P
	AVH NOS	P	P	N	N N
20	AVH NOS	P/N	P	N	N P
	AVH PTVH	P/N	P	P	P P
	AVH NOS	N	P	P	P P/N
	AVH IVDA	N	P	N	N P
25	AVH NOS	N	P/N	N	N N
	AVH NOS	P	P	N	N P
	AVH PTVH	N	P	P	P P
30	crypto	P	P	P	P P
	CVH NOS	N	P	P	P P
	CVH NOS	N	N	N	N N
	AVH PTVH	N	P	P(+)	P(++) N
35	AVH PTVH	N	P/N	P(+)	P(++) P
	AVH PTVH	N	P/N	P(+)	P(++) P
	CVH IVDA	P	P	P	P P
40	CVH IVDA	P	P	P	P P
	CVH IVDA	P	P	P	P P
	CVH IVDA	P	P	P	P P
	AVH NOS	N	P	N	N N
45	CVH IVDA	P	P	P	P P/N
	AVH IVDA	P	P	P	P N
	AVH NOS	P	P	N	N N
50	AVH NOS	P	P	N	N N
	CVH PTVH	P	P	N	N P/N

<u>INDIVIDUAL</u>		<u>ANTIGEN</u>			
		<u>S2</u>	<u>C22</u>	<u>C100</u>	<u>C33c</u> <u>NS5</u>
5	AVH PTVH	N	P	N	P P
	AVH NOS	N	N	N	N N
	AVH NOS	N	P	N	N N
	AVH NOS	P	N	N	N N
10	CVH NOS	N	N	N	N N
	AVH NOS	N	P/N	N	N N
	AVH IVDA	N	P	P	P P
15	crypto	N	P	N	N P/N
	crypto	P	P	P/N	P P
	AVH IVDA	N	N	P	P N
	AVH IVDA	N	P	P	P N
20	AVH NOS	N	N	N	N N
	AVH NOS	N	N	N	N N
	CVH IVDA	P	P	P	P P
25	CVH PTVH	N	N	N	N N
	CVH PTVH	P	P	P(+)	P(+++) P
	CVH PTVH	P	P	P(+)	P(+++) P
	CVH NOS	P/N	N	N	N N
30	CVH NOS	N	N	N	N N
	CVH PTVH	P	P	P	P P
	CVH PTVH	P	P	P	P P
35	CVH PTVH	P	P	P	P P
	AVH IVDA	N	P	P	P P
	CVH NOS	N	N	N	N N
40	CVH NOS	N	N	N	N N
	CVH PTVH	P	P	P	P P
	AVH NOS	P	P	N	N P/N
	AVH NOS	N	P/N	N	N N
45	CVH PTVH	P	P	N	N P
	CVH NOS	N	P/N	N	N N
	AVH NOS	N	P	N	N N
50	AVH NOS	N	P	N	N N
	CVH PTVH	N	P	N	N N

55

<u>INDIVIDUAL</u>		<u>ANTIGEN</u>			
		<u>S2</u>	<u>C22</u>	<u>C100</u>	<u>C33c</u> <u>NS5</u>
5	AVH IVDA	N	P	N	P P
	AVH NOS	P	N	N	N N
	CVH NOS	N	N	N	N N
10	CVH NOS	N	N	N	N N
	CVH IVDA	P	P	P	P P
	CVH IVDA	P/N	P	P	P P
	CVH IVDA	P	P	P	P P
15	CVH IVDA	N	P	P	P P
	AVH NOS	N	P	N	N N
	CVH IVDA	N	P	N	N P
20	CVH IVDA	N	P	N	N P
	AVH PTVH	P	P	N	P P
	AVH PTVH	P	P	N	P P
	CVH NOS	N	P/N	N	N P/N
25	CVH NOS	N	P	N	N N
	CVH NOS	N	N	N	N N
	CVH PTVH	P	P	P	P P
30	CVH PTVH	P	P	P	P P
	CVH PTVH	P	P	P	P P
	AVH IVDA	N	P	N	N P
	AVH IVDA	N	P	P(++)	P(+) P
35	CVH PTVH	P	P	P	P P
	AVH PTVH	N	P	P	P P
	CVH PTVH?	N	P	P	P P
40	CVH PTVH?	P/N	P	P	P P
	CVH NOS HS	P	P	N	N N
	CVH IVDA	P	P	P	P N
45	CVH PTVH	N	P	P	P P
	CVH PTVH	P	P	P	P P/N
	CVH NOS	P	P	P	P P
	CVH IVDA	P	P	P	P P
50	CVH PTVH	P	P	P	P N
	CVH PTVH	P	P	P	P P

55

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<u>INDIVIDUAL</u>		<u>ANTIGEN</u>				
		<u>S2</u>	<u>C22</u>	<u>C100</u>	<u>C33c</u>	<u>NS5</u>
5	CVH NOS	N	N	N	N	P/N
	CVH NOS	N	P/N	N	N	P/N
	CVH PTVH	P	P	P	P	P
	CVH NOS	N	P	N	N	N
10	CVH NOS	N	N	N	N	N
	CVH NOS	P	P	N	N	P/N
	CVH NOS	N	N	N	N	N
15	CVH NOS HS	P	P	P	P	P
	CVH NOS HS	P	P	P	P	P
	CVH PTVH	N	N	N	N	N
	AVH PTVH	N	P	P	P	P
20	AVH NOS			-	-	
	CVH PTVH	N	P	P/N	P(+++)	N
	crypto	P	P	P	P	P
25	crypto	P	P	P	P	P
	crypto	N	P	N	N	N
	crypto	N	P	P	P	P
	CVH PTVH	P	P	P	P	P
30	crypto	N	N	N	N	N
	crypto	N	P	N	N	P/N
	crypto	N	P	N	N	P
35	crypto	P	P	P	P	P
	crypto	N	P	N	P	N
	crypto			-	-	
	crypto			-	-	
40	CVH NOS			-	-	
	AVH-IVDA	N	P	N	P(+)	P

45

50

55

INDIVIDUALANTIGEN

	<u>S2</u>	<u>C22</u>	<u>C100</u>	<u>C33c</u>	<u>NS5</u>
5 AVH-IVDA	N	P/N	N	P(++)	N

AVH = acute viral hepatitis

CVH = chronic viral hepatitis

10 PTVH = post-transfusion viral hepatitis

IVDA = intravenous drug abuser

crypto = cryptogenic hepatitis

15 NOS = non-obvious source

P = positive

N = negative

20 Per these results, no single antigen reacted with all sera. C22 and C33c were the most reactive and S2 reacted with some sera from some putative acute HCV cases with which no other antigen reacted. Based on these results, the combination of two antigens that would provide the greatest range of detection is C22 and C33c. If one wished to detect a maximum of acute infections, S2 would be included in the combination.

Table 2 below presents the results of the testing on the paid blood donors.

25 Table 2

		<u>Antigens</u>				
	<u>Donor</u>	<u>C100</u>	<u>C33c</u>	<u>C22</u>	<u>S2</u>	<u>NS5</u>
30	1	N	N	N	N	N
	2	N	N	N	N	N
	3	P	P	P	P	P
35	4	N	N	N	N	N
	5	N	N	N	N	N
	6	N	N	N	N	N
	7	N	N	N	N	N
40	8	N	N	N	N	N

45

50

55

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		Antigens				
	<u>Donor</u>	<u>C100</u>	<u>C33c</u>	<u>C22</u>	<u>S2</u>	<u>NS5</u>
5	9	N	N	N	N	N
	10	N	N	N	N	N
	11	N	N	N	N	N
	12	N	N	N	N	N
10	13	N	N	N	N	N
	14	N	N	N	N	N
	15	N	N	N	N	N
15	16	N	N	N	N	N
	17	N	N	N	N	N
	18	P	P	P	P	P
	19	P	P	N	P	P
20	20	P	P	N	P	P
	21	N	N	N	N	N
	22	N	P	P	N	P
25	23	P	P	P	P	P
	24	N	N	N	N	N
	25	N	N	N	N	N
	26	N	N	N	N	N
30	27	N	N	N	N	N
	28	N	N	N	N	N
	29	N	N	N	N	N
35	30	N	N	N	N	N
	31	P	P	P	N	P
	32	N	N	N	N	N
	33	N	N	N	N	N
40	34	N	N	N	N	P
	35	N	N	P	N	P
	36	N	N	N	N	N
45	37	N	N	N	N	N
	38	N	N	N	N	N
	39	N	N	N	N	N
50	40	N	N	N	N	N
	41	N	N	N	N	P
	42	N	N	N	N	N
55						

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		Antigens				
	<u>Donor</u>	<u>C100</u>	<u>C33c</u>	<u>C22</u>	<u>S2</u>	<u>NS5</u>
5	43	N	N	N	N	N
	44	N	N	N	N	N
	45	N	N	N	N	N
	46	N	N	N	N	N
10	47	P	P	N	N	P
	48	N	N	N	N	N
	49	N	N	N	N	N
	50	N	N	N	N	N
15	51	N	P	P	N	P
	52	N	N	N	N	N
	53	N	P	P	N	P
	54	P	P	P	P	N
20	55	N	N	N	N	N
	56	N	N	N	N	N
	57	N	N	N	N	N
	58	N	N	N	N	N
25	59	N	N	N	N	N
	60	N	N	N	N	N
	61	N	N	N	N	N
	62	N	N	N	N	N
30	63	N	N	N	N	N
	64	N	N	N	N	N
	65	N	N	N	N	N
	66	N	N	N	N	N
35	67	N	N	N	N	N
	68	N	N	N	N	N
	69	N	N	N	N	N
	70	P	P	P	P	P
40	71	N	N	N	N	N
	72	N	N	N	N	N
	73	P	P	P	P	N
	74	N	N	N	N	N
45	75	N	N	N	N	N
	76	N	N	N	N	P

55

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Antigens						
	<u>Donor</u>	<u>C100</u>	<u>C33c</u>	<u>C22</u>	<u>S2</u>	<u>NS5</u>
5	77	N	N	N	N	N
	78	N	N	N	N	N
	79	N	N	N	N	N
	80	N	N	N	N	N
10	81	N	N	N	N	N
	82	N	N	N	N	N
	83	P	P	N	N	N
15	84	N	N	P	N	N
	85	N	N	N	N	N
	86	P	P	P	P	N
	87	N	N	N	N	N
20	88	N	N	N	N	N
	89	P	P	P	P	P
	90	P	P	P	P	N
25	91	N	N	N	N	P
	92	P	P	P	N	N
	93	N	N	N	N	N
	94	N	N	N	N	N
30	95	N	N	N	N	N
	96	N	N	N	N	N
	97	N	N	N	N	N
35	98	N	P	P	P	P
	99	P	P	P	P	P
	100	N	N	N	N	N
40	101	P	P	P	P	P
	102	N	N	N	N	N
	103	N	N	N	N	N
	104		N	N	N	N
45	105	P	P	P	P	N
	106	N	N	N	N	N
	107	N	N	N	N	N
50	108	N	N	N	N	N
	109	P	P	P	P	P
	110	P	P	P	N	P
55						

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Antigens						
	<u>Donor</u>	<u>C100</u>	<u>C33c</u>	<u>C22</u>	<u>S2</u>	<u>NS5</u>
5	111	P	P	P	N	P
	112	N	N	N	N	N
	113	P	P	P	P	P
	114	N	N	N	N	N
10	115	N	N	N	N	N
	116	P	P	P	P	P
	117	N	N	N	N	N
15	118	N	N	N	N	N
	119	N	N	N	N	N
	120	P	P	P	P	P
	121	N	N	N	N	N
20	122	N	P	P	N	P
	123	N	N	N	N	N
	124	N	N	N	N	N
25	125	N	N	N	N	N
	126	P	N	N	N	N
	127	N	N	N	N	N
	128	N	N	N	N	N
30	129	N	N	N	N	N
	130	P	P	P	P	N
	131	N	N	N	N	P
35	132	N	N	N	N	N
	133	N	N	N	N	N
	134	N	N	N	N	N
40	135	N	N	N	N	N
	136	N	N	N	N	N
	137	N	N	N	N	N
	138	N	N	N	N	N
45	139	N	N	N	N	N
	140	P	N	N	N	N
	141	P	N	P	P	P
50	142	N	N	N	N	N
	143	N	N	N	N	N
	144	N	N	N	N	N

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Antigens						
	<u>Donor</u>	<u>C100</u>	<u>C33c</u>	<u>C22</u>	<u>S2</u>	<u>NS5</u>
5	145	N	N	N	N	N
	146	N	N	N	N	N
	147	N	N	N	N	N
	148	N	N	N	N	N
10	149	N	N	N	N	N
	150	N	N	N	N	N
	151	N	N	N	N	N
15	152	N	N	N	N	N
	153	N	N	N	N	N
	154	P	P	P	P	P
	155	N	N	N	N	N
20	156	N	N	N	N	N
	157	N	N	N	N	N
	158	N	N	N	N	N
25	159	N	N	N	N	N
	160	N	N	N	N	N
	161	P	P	P	P	P
30	162	N	N	N	N	N
	163	N	N	N	N	N
	164	P	P	P	N	P
	165	N	N	N	N	N
35	166	P	P	P	N	P
	167	N	N	N	N	N
	168	N	N	N	N	N
40	169	N	N	N	N	N
	170	N	N	N	N	N
	171	N	N	N	N	N
	172	N	N	N	N	N
45	173	N	N	N	N	N
	174	N	N	N	N	N
	175	N	N	N	N	N
50	176	N	N	N	N	N
	177	N	N	N	N	P
	178	N	N	N	N	N
55						

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Antigens						
	Donor	C100	C33c	C22	S2	NS5
5	179	N	N	N	N	N
	180	N	N	N	N	N
	181	N	N	N	N	N
	182	N	N	N	N	N
10	183	P	P	P	P	P
	184	N	N	N	N	N
	185	N	N	N	N	N
	186	N	N	N	N	N
15	187	N	N	N	N	N
	188	N	P	P	N	N
	189	N	N	N	N	N
	190	N	N	N	N	N
20	191	N	N	N	N	N
	192	N	N	N	N	N
	193	N	N	N	N	N
	194	N	N	N	N	N
25	195	N	N	N	N	N
	196	N	N	N	N	N
	197	N	N	N	N	P
	198	P	P	P	N	N
30	199	N	N	N	N	P
	200	P	P	P	P	N

35 The results on the paid donors generally confirms the results from the sera of infected individuals.

Example 7: ELISA Determinations of HCV Antibodies Using Combination of HCV Antigens

40 Plates coated with a combination of C22 and C33c antigens are prepared as follows. A solution containing coating buffer (50mM Na Borate, pH 9.0), 21 ml/plate, BSA (25 micrograms/ml), C22 and C33c (2.50 micrograms/ml each) is prepared just prior to addition to the Removeawell Immulon I plates (Dynatech Corp.). After mixing for 5 minutes, 0.2ml/well of the solution is added to the plates, they are covered and incubated for 2 hours at 37°C, after which the solution is removed by aspiration. The wells are washed once with 400 microliters wash buffer (100 mM sodium phosphate, pH 7.4, 140 mM sodium chloride, 0.1% (W/V) casein, 1% (W/V) Triton x-100(TM), 0.01% (W/V) Thimerosal).

45 After removal of the wash solution, 200 microliters/well of Postcoat solution (10 mM sodium phosphate, pH 7.2, 150 mM sodium chloride, 0.1% (w/v) casein, 3% sucrose and 2 mM phenylmethylsulfonylfluoride (PMSF)) is added, the plates are loosely covered to prevent evaporation, and are allowed to stand at room temperature for 30 minutes. The wells are then aspirated to remove the solution, and lyophilized dry overnight, without shelf heating. The prepared plates may be stored at 2-8°C in sealed aluminum pouches with dessicant (3 g Sorb-it™ packs).

50 In order to perform the ELISA determination, 20 microliters of serum sample or control sample is added to a well containing 200 microliters of sample diluent (100 mM sodium phosphate, pH 7.4, 500 mM sodium chloride, 1 mM EDTA, 0.1% (W/V) Casein, 0.01% (W/V) Thimerosal, 1% (W/V) Triton X-100(TM), 100 micrograms/ml yeast extract). The plates are sealed, and are incubated at 37°C for two hours, after which the solution is removed by aspiration, and the wells are washed three times with 400 microliters of wash buffer -(phosphate buffered saline (PBS) containing 0.05% Tween 20(TM)). The washed wells are treated with 200 microliters of mouse anti-human IgG-horse radish peroxidase (HRP) conjugate contained in a solution of Ortho conjugate diluent (10 mM sodium phosphate, pH 7.2, 150 mM sodium chloride, 50% (V/V) fetal bovine serum, 1% (V/V) heat treated horse serum, 1 mM K₃Fe(CN)₆, 0.05% (W/V) Tween 20, 0.02% (W/V) Thimerosal). Treatment is for 1 hour at 37°C, the solution is removed by aspiration, and

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the wells are washed three times with 400 ml wash buffer, which is also removed by aspiration. To determine the amount of bound enzyme conjugate, 200 microliters of substrate solution (10 mg O-phenylenediamine dihydrochloride per 5 ml of Developer solution) is added. Developer solution contains 50 mM sodium citrate adjusted to pH 5.1 with phosphoric acid, and 0.6 microliters/ml of 30% H₂O₂. The plates containing the substrate solution are incubated in the dark for 30 minutes at room temperature, the reactions are stopped by the addition of 50 microliters/ml 4N sulfuric acid, and the ODs determined.

In a similar manner, ELISAs using fusion proteins of C22 and C33c, and C22, C33c, and S2 and combinations of C22 and C100, C22 and S2, C22 and an NS5 antigen, C22, C33c, and S2, and C22, C100, and S2 may be carried out.

Claims

Claims for the following Contracting States : AT, BE, CH, LI, DE, DK, FR, GR, IT, LU, NL, SE

1. A combination of hepatitis C virus (HCV) epitope sequences in one or more polypeptides made by chemical synthesis or recombinant expression, immobilized on the surface of a solid matrix suitable for detection of HCV by immunoassay, comprising:

- (a) a first epitope sequence from the C domain of the HCV polyprotein;
- (b) a second epitope sequence from a second domain of the HCV polyprotein which domain is:

- (i) the NS3 domain of the HCV polyprotein;
- (ii) the NS4 domain of the HCV polyprotein; or
- (iii) the NS5 domain of the HCV polyprotein; and

- (c) a third epitope sequence from a third domain of the HCV polyprotein which domain is:

- (i) the NS3 domain of the HCV polyprotein;
- (ii) the NS4 domain of the HCV polyprotein; or
- (iii) the NS5 domain of the HCV polyprotein;

wherein the third domain is different from the second domain;
with the proviso that the combination is not the peptide p1 with C100-3, the peptide p35 with C100-3 or the peptide p99 with C100-3.

2. A combination according to claim 1 wherein the second domain is NS3.

3. A combination according to claim 1 wherein the second domain is NS4.

4. A combination according to claim 1 which comprises:

- (a) a first epitope sequence from the C domain of the HCV polyprotein;
- (b) a second epitope sequence from the NS3 domain of the HCV polyprotein; and
- (c) a third epitope sequence from the NS4 domain of the HCV polyprotein.

5. A combination according to any one of claims 1 to 4 wherein the solid matrix is the surface of a microtiter plate well, a bead or dipstick.

6. A combination according to any one of claims 1 to 5 wherein the first, second and third epitope sequences are contained in first, second and third polypeptides respectively individually bound to the solid matrix.

7. A combination according to claim 6 wherein the solid matrix is a dipstick and the polypeptides are distributed individually in a pattern such that binding to the first, second and third polypeptides may be discerned.

8. The combination of any one of claims 1 to 5 wherein the combination is in the form of a fusion polypeptide.

9. A method for detecting antibodies to hepatitis C virus (HCV) in a mammalian body component suspected of con-

taining said antibodies comprising contacting said body component with the combination of any one of claims 1 to 8 under conditions that permit antibody-antigen reaction and detecting the presence of immune complexes of said antibodies and said polypeptide epitope sequences.

- 5 10. A kit for carrying out an assay for detecting antibodies to hepatitis C antigen (HCV) in a mammalian body component suspected of containing said antibodies comprising in packaged combination:

- (a) the combination of HCV epitope sequences of any one of claims 1 to 8,
 (b) standard control reagents; and
 10 (c) instructions for carrying out the assay.

Claims for the following Contracting State : ES

- 15 1. A method for detecting antibodies to hepatitis C virus (HCV) in a mammalian body component suspected of containing said antibodies comprising contacting said body component with the combination of polypeptide HCV epitope sequences under conditions that permit antibody-antigen reaction and detecting the presence of immune complexes of said antibodies and said polypeptide epitope sequences, wherein epitope sequences are in one or more polypeptides made by chemical synthesis or recombinant expression, immobilized on the surface of a solid matrix suitable for detection of HCV by immunoassay, comprising:

- (a) a first epitope sequence from the C domain of the HCV polyprotein;
 (b) a second epitope sequence from a second domain of the HCV polyprotein which domain is:

- 25 (i) the NS3 domain of the HCV polyprotein;
 (ii) the NS4 domain of the HCV polyprotein; or
 (iii) the NS5 domain of the HCV polyprotein; and

- (c) a third epitope sequence from a third domain of the HCV polyprotein which domain is:

- 30 (i) the NS3 domain of the HCV polyprotein;
 (ii) the NS4 domain of the HCV polyprotein; or
 (iii) the NS5 domain of the HCV polyprotein;

35 wherein the third domain is different from the second domain;
 with the proviso that the combination is not the peptide p1 with C100-3, the peptide p35 with C100-3 or the peptide p99 with C100-3.

- 40 2. A method according to claim 1 wherein the second domain is NS3.

3. A method according to claim 1 wherein the second domain is NS4.

4. A method according to claim 1 in which the combination comprises:

- 45 (a) a first epitope sequence from the C domain of the HCV polyprotein;
 (b) a second epitope sequence from the NS3 domain of the HCV polyprotein; and
 (c) a third epitope sequence from the NS4 domain of the HCV polyprotein.

- 50 5. A method according to any one of claims 1 to 4 wherein the solid matrix is the surface of a microtiter plate well, a bead or dipstick.

6. A method according to any one of claims 1 to 5 wherein the first, second and third epitope sequences are contained in first, second and third polypeptides respectively individually bound to the solid matrix.

- 55 7. A method according to claim 6 wherein the solid matrix is a dipstick and the polypeptides are distributed individually in a pattern such that binding to the first, second and third polypeptides may be discerned.

8. The method of any one of claims 1 to 5 wherein the combination is in the form of a fusion polypeptide.

Patentansprüche

Patentansprüche für folgende Vertragsstaaten : AT, BE, CH, DE, DK, FR, GR, IT, LI, LU, NL, SE

- 5 1. Kombination von Hepatitis-C-Virus-(HCV-)Epitopsequenzen in einem oder mehreren Polypeptid(en), hergestellt durch chemische Synthese oder rekombinante Expression, immobilisiert auf der Oberfläche einer festen Matrix, mit der Eignung zum Nachweis von HCV in einem Immunoassay, umfassend:
 - 10 (a) eine erste Epitopsequenz aus der C-Domäne des HCV-Polypeptins;
 - (b) eine zweite Epitopsequenz aus einer zweiten Domäne des HCV-Polypeptins, wobei die Domäne:
 - 15 (i) die NS3-Domäne des HCV-Polypeptins;
 - (ii) die NS4-Domäne des HCV-Polypeptins; oder
 - (iii) die NS5-Domäne des HCV-Polypeptins ist; und
 - (c) eine dritte Epitopsequenz aus einer dritten Domäne des HCV-Polypeptins, wobei die Domäne:
 - 20 (i) die NS3-Domäne des HCV-Polypeptins;
 - (ii) die NS4-Domäne des HCV-Polypeptins; oder
 - (iii) die NS5-Domäne des HCV-Polypeptins ist;

wobei die dritte Domäne sich von der zweiten Domäne unterscheidet;
mit der Maßgabe, daß die Kombination nicht das Peptid pl mit C100-3, das Peptid p35 mit C100-3 oder das Peptid p99 mit C100-3 ist.

- 2. Kombination nach Anspruch 1, worin die zweite Domäne NS3 ist.
- 3. Kombination nach Anspruch 1, worin die zweite Domäne NS4 ist.
- 30 4. Kombination nach Anspruch 1, umfassend:
 - (a) eine erste Epitopsequenz aus der C-Domäne des HCV-Polypeptins;
 - (b) eine zweite Epitopsequenz aus der NS3-Domäne des HCV-Polypeptins; und
 - 35 (c) eine dritte Epitopsequenz aus der NS4-Domäne des HCV-Polypeptins.
- 5. Kombination nach einem der Ansprüche 1 bis 4, worin die feste Matrix die Oberfläche einer Kavität einer Mikrotiterplatte, eines Kügelchens oder eines Tauchstäbchens ist.
- 40 6. Kombination nach einem der Ansprüche 1 bis 5, worin die ersten, zweiten und dritten Epitopsequenzen in den ersten, zweiten bzw. dritten Polypeptiden in individueller Bindung an die feste Matrix enthalten sind.
- 7. Kombination nach Anspruch 6, worin die feste Matrix ein Tauchstäbchen ist und die Polypeptide individuell in einem Muster so verteilt sind, daß die Bindung an das erste, zweite und dritte Polypeptid unterschieden werden kann.
- 45 8. Kombination nach einem der Ansprüche 1 bis 5, worin die Kombination in Form eines Fusions-Polypeptids vorliegt.
- 9. Verfahren zum Nachweis von Antikörpern gegen das Hepatitis-C-Virus (HCV) in einer Körperkomponente eines Säugers, der vermutlich die Antikörper enthält, wobei man die Körperkomponente mit der Kombination nach einem der Ansprüche 1 bis 8 unter Bedingungen in Kontakt bringt, die eine Antikörper-Antigen-Reaktion erlauben, und die Anwesenheit von Immunkomplexen aus den Antikörpern und den Polypeptid-Epitopsequenzen nachweist.
- 50 10. Kit zur Durchführung eines Assays zum Nachweis von Antikörpern gegen das Hepatitis-C-Antigen (HCV) in einer Körperkomponente eines Säugers, der vermutlich die Antikörper enthält, umfassend in abgepackter Kombination:
 - 55 (a) die Kombination aus HCV-Epitopsequenzen nach einem der Ansprüche 1 bis 9;
 - (b) Standard-Kontrollreagentien; und
 - (c) Anweisungen zur Durchführung des Assays.

Patentansprüche für folgenden Vertragsstaat : ES

1. Verfahren zum Nachweis von Antikörpern gegen das Hepatitis-C-virus (HCV) in einer Körperkomponente eines Säugers, der vermutlich die Antikörper enthält, wobei man die Körperkomponente mit der Kombination von Polypeptid-HCV-Epitopsequenzen unter Bedingungen in Kontakt bringt, die eine Antikörper-Antigen-Reaktion erlauben, und die Anwesenheit von Immunkomplexen aus den Antikörpern und den Polypeptid-Epitopsequenzen nachweist, wobei die Epitopsequenzen in einem oder mehreren Polypeptid(en) vorhanden sind, die durch chemische Synthese oder rekombinante Expression hergestellt wurden, auf der Oberfläche einer festen Matrix immobilisiert sind und zum Nachweis von HCV durch einen Immunoassay geeignet sind, umfassend:
 - (a) eine erste Epitopsequenz aus der C-Domäne des HCV-Polypeptins;
 - (b) eine zweite Epitopsequenz aus einer zweiten Domäne des HCV-Polypeptins, wobei die Domäne:
 - (i) die NS3-Domäne des HCV-Polypeptins;
 - (ii) die NS4-Domäne des HCV-Polypeptins; oder
 - (iii) die NS5-Domäne des HCV-Polypeptins ist; und
 - (c) eine dritte Epitopsequenz aus einer dritten Domäne des HCV-Polypeptins, wobei die Domäne:
 - (i) die NS3-Domäne des HCV-Polypeptins;
 - (ii) die NS4-Domäne des HCV-Polypeptins; oder
 - (iii) die NS5-Domäne des HCV-Polypeptins ist;wobei die dritte Domäne sich von der zweiten Domäne unterscheidet; mit der Maßgabe, daß die Kombination nicht das Peptid p1 mit C100-3, das Peptid p35 mit C100-3 oder das Peptid p99 mit C100-3 ist.
2. Verfahren nach Anspruch 1, wobei die zweite Domäne NS3 ist.
3. Verfahren nach Anspruch 1, wobei die zweite Domäne NS4 ist.
4. Verfahren nach Anspruch 1, wobei die Kombination umfaßt:
 - (a) eine erste Epitopsequenz aus der C-Domäne des HCV-Polypeptins;
 - (b) eine zweite Epitopsequenz aus der NS3-Domäne des HCV-Polypeptins; und
 - (c) eine dritte Epitopsequenz aus der NS4-Domäne des HCV-Polypeptins.
5. Verfahren nach einem der Ansprüche 1 bis 4, wobei die feste Matrix die Oberfläche einer Kavität einer Mikrotiterplatte, eines Kügelchens oder eines Tauchstäbchens ist.
6. Verfahren nach einem der Ansprüche 1 bis 5, wobei die ersten, zweiten und dritten Epitopsequenzen in den ersten, zweiten bzw. dritten Polypeptiden in individueller Bindung an die feste Matrix enthalten sind.
7. Verfahren nach Anspruch 6, wobei die feste Matrix ein Tauchstäbchen ist und die Polypeptide individuell in einem Muster so verteilt sind, daß die Bindung an das erste, zweite und dritte Polypeptid unterschieden werden kann.
8. Verfahren nach einem der Ansprüche 1 bis 5, wobei die Kombination in Form eines Fusions-Polypeptids vorliegt.

Revendications

Revendications pour les Etats contractants suivants : AT, BE, CH, DE, DK, FR, GR, IT, LI, LU, NL, SE

1. Combinaison de séquences épitopiques de virus de l'hépatite C (HCV) dans un ou plusieurs polypeptides produits par synthèse chimique ou par expression recombinante, immobilisée à la surface d'une matrice solide appropriée pour la détection du HCV par test immunologique, comprenant :

- (a) une première séquence épitopique du domaine C de la polyprotéine de HCV ;
- (b) une deuxième séquence épitopique d'un deuxième domaine de la polyprotéine de HCV, domaine qui est :
- 5 (i) le domaine NS3 de la polyprotéine de HCV ;
(ii) le domaine NS4 de la polyprotéine de HCV ; ou
(iii) le domaine NS5 de la polyprotéine de HCV ; et
- (c) une troisième séquence épitopique d'un troisième domaine de la polyprotéine de HCV, domaine qui est :
- 10 (i) le domaine NS3 de la polyprotéine de HCV ;
(ii) le domaine NS4 de la polyprotéine de HCV ; ou
(iii) le domaine NS5 de la polyprotéine de HCV ;
- 15 le troisième domaine étant différent du deuxième domaine ;
avec la condition que la combinaison ne soit pas le peptide p1 avec C100-3, le peptide p35 avec C100-3 ou le peptide p99 avec C100-3.
2. Combinaison selon la revendication 1, dans laquelle le deuxième domaine est NS3.
- 20 3. Combinaison selon la revendication 1, dans laquelle le deuxième domaine est NS4.
4. Combinaison selon la revendication 1, qui comprend :
- 25 (a) une première séquence épitopique du domaine C de la polyprotéine de HCV,
(b) une deuxième séquence épitopique du domaine NS3 de la polyprotéine de HCV ; et
(c) une troisième séquence épitopique du domaine NS4 de la polyprotéine de HCV.
- 30 5. Combinaison selon l'une quelconque des revendications 1 à 4, dans laquelle la matrice solide est la surface d'un puits de plaque de microtitration, d'une bille ou d'une bandelette réactive.
6. Combinaison selon l'une quelconque des revendications 1 à 5, dans laquelle les première, deuxième et troisième séquences épitopiques sont contenues dans les premier, deuxième et troisième polypeptides respectivement, liés individuellement à la matrice solide.
- 35 7. Combinaison selon la revendication 6, dans laquelle la matrice solide est une bandelette réactive et les polypeptides sont distribués individuellement dans une disposition telle que les liaisons avec les premier, deuxième et troisième polypeptides puissent être distinguées.
- 40 8. Combinaison selon l'une quelconque des revendications 1 à 5, dans laquelle la combinaison est sous la forme d'un polypeptide de fusion.
9. Méthode pour détecter des anticorps dirigés contre le virus de l'hépatite C (HCV) dans un constituant corporel d'un mammifère dont on soupçonne qu'il contient lesdits anticorps, comprenant les étapes consistant à mettre en contact ledit constituant corporel avec la combinaison de l'une quelconque des revendications 1 à 8 dans des conditions qui permettent une réaction antigène-anticorps et à détecter la présence de complexes immuns desdits anticorps et desdites séquences polypeptidiques épitopiques.
- 50 10. Kit pour effectuer un test de détection d'anticorps dirigés contre un antigène de l'hépatite C (HCV) dans un constituant corporel d'un mammifère dont on soupçonne qu'il contient lesdits anticorps, comprenant dans un ensemble conditionné :
- 55 (a) la combinaison de séquences épitopiques de HCV de l'une quelconque des revendications 1 à 8;
b) des réactifs témoins étalons ; et

(c) des instructions pour effectuer le test.

Revendications pour l'Etat contractant suivant : ES

- 5
1. Méthode pour détecter des anticorps du virus de l'hépatite C (HCV) dans un constituant corporel d'un mammifère dont on soupçonne qu'il contient lesdits anticorps, comprenant les étapes consistant à mettre en contact ledit constituant corporel avec la combinaison de séquences polypeptidiques épitopiques de HCV, dans des conditions qui permettent une réaction antigène-anticorps, et à détecter la présence de complexes immuns desdits anticorps et desdites séquences polypeptidiques épitopiques, dans laquelle les séquences épitopiques sont dans un ou plusieurs polypeptides produits par synthèse chimique ou expression recombinante, immobilisés à la surface d'une matrice solide appropriée pour la détection de HCV par un test immunologique, comprenant :
 - 10
(a) une première séquence épitopique du domaine C de la polyprotéine de HCV ;
 - 15
(b) une deuxième séquence épitopique d'un deuxième domaine de la polyprotéine de HCV, domaine qui est :
 - (i) le domaine NS3 de la polyprotéine de HCV ;
 - (ii) le domaine NS4 de la polyprotéine de HCV ; ou
 - 20
(iii) le domaine NS5 de la polyprotéine de HCV ; et
 - (c) une troisième séquence épitopique d'un troisième domaine de la polyprotéine de HCV, domaine qui est :
 - 25
(i) le domaine NS3 de la polyprotéine de HCV ;
 - (ii) le domaine NS4 de la polyprotéine de HCV ; ou
 - (iii) le domaine NS5 de la polyprotéine de HCV ;

le troisième domaine étant différent du deuxième domaine ;
avec la condition que la combinaison ne soit pas le peptide p1 avec C100-3, le peptide p35 avec C100-3 ou
30 le peptide p99 avec C100-3.
2. Méthode selon la revendication 1, dans laquelle le deuxième domaine est NS3.
3. Méthode selon la revendication 1, dans laquelle le deuxième domaine est NS4.
- 35
4. Méthode selon la revendication 1, dans laquelle la combinaison comprend :
 - (a) une première séquence épitopique du domaine C de la polyprotéine de HCV ;
 - 40
(b) une deuxième séquence épitopique du domaine NS3 de la polyprotéine de HCV ; et
 - (c) une troisième séquence épitopique du domaine NS4 de la polyprotéine de HCV.
- 45
5. Méthode selon l'une quelconque des revendications 1 à 4, dans laquelle la matrice solide est la surface d'un puits de plaque de microtitration, d'une bille ou d'une bandelette réactive.
6. Méthode selon l'une quelconque des revendications 1 à 5, dans laquelle les première, deuxième et troisième séquences d'épitope sont contenues dans les premier, deuxième et troisième polypeptides respectivement, liés individuellement à la matrice solide.
- 50
7. Méthode selon la revendication 6, dans laquelle la matrice solide est une bandelette réactive et les polypeptides sont distribués individuellement dans une disposition telle que les liaisons aux premier, deuxième et troisième polypeptides puissent être distinguées.
- 55
8. Méthode selon l'une quelconque des revendications 1 à 5, dans laquelle la combinaison est sous la forme d'un polypeptide de fusion.

FIG. 1A

-319 CACTCCACCATGAATCACTCCCTGTGAGAACTACTGTCTTACGCAGAAAGCGTCTAG
 GTGAGGTGGTACTTAGTGAGGGGACACTCCTTGATGACAGAAAGTGGTCTTTCGCAGATC
 -259 CCATGGCGTTAGTATGAGTGTGTCGTCAGCCTCCAGGACCCCCCTCCCGGGAGAGCCATA
 GGTACCGCAATCATACTACAGCACGTCGGAGGTCCTGGGGGGAGGCCCTCTCGGTAT
 -199 GTGGTCTGCGGAACCGGTGAGTACACCGGAATTGCCAGGACGACCGGGTCTTCTTGGGA
 CACCAGACGCCTTGGCCACTCATGTGGCCTTAACGGTCTCTGGCCCCAGGAAAGAACCT
 -139 TCAACCCGCTCAATGCCCTGGAGATTGGGGCGTGCCCCCGCAAGACTGTAGCCGAGTAGT
 AGTTGGGCGAGTTACGGACCTCTAAACCCGACGGGGCGTCTGACGATCGGCTCATCA
 - 79 GTTGGGTCGCGAAAGGCCTTGTGGTACTGCCCTGATAGGGTGCTTGCAGTGCCCCGGGAG
 CAACCCAGCGCTTTCGGAACACACCATGACGGACTATCCACGAACGCTCACGGGGCCCTC
 - 19 GTCTCGTAGACCGTGCAAC
 CAGAGCATCTGGCACGTGG

Arg Thr

1 MetSerThrAsnProLysProGlnLysLysAsnLysArgAsnThrAsnArgArgProGln
 ATGAGCACGAATCCTAAACCTCAAAAAAACAACGTAACACCAACCGTCGCCACAG
 TACTCGTGCTTAGGATTGGAGTTTTTTGTGTCATTGTGGTTGGCAGCGGGTGTG

61 AspValLysPheProGlyGlyGlyGlnIleValGlyGlyValTyrLeuLeuProArgArg
 GACGTCAAGTTCCTGGGTGGCGGTGAGTCTGTTGGAGTTTACTTGTGTCGCCGAGG
 CTGCAGTTCAAGGGCCACCGCCAGTCTAGCAACCACTCAAAATGAACAACGGCGGTCC

121 GlyProArgLeuGlyValArgAlaThrArgLysThrSerGluArgSerGlnProArgGly
 GGCCCTAGATTGGGTGTCGGCGCACGAGAAAGACTCCGAGCGTCCGAAACCTCGAGGT
 CCGGGATCTAACCACACACGCGCGTCTTTCTGAAGGCTCGCCAGCGTTGGAGCTCCA

 181 ArgArgGlnProIleProLysAlaArgArgProGluGlyArgThrTrpAlaGlnProGly
 AGACGTCAGCCTATCCCCAAGGCTCGTCGGCCCGAGGCGAGGACCTGGGCTCAGCCCCGG
 TCTGCAGTCGGATAGGGTTCCGAGCAGCCGGGCTCCCGTCTGGACCCGAGTCGGGCCC

 241 TyrProTrpProLeuTyrGlyAsnGluGlyCysGlyTrpAlaGlyTrpLeuLeuSerPro
 TACCCTTGGCCCCCTCTATGGCAATGAGGGCTGCGGGTGGCGGATGGCTCCTGTCTCCC
 ATGGAAACCGGGAGATACCGTTACTCCCGACGCCACCCGCCCTACCGAGGACAGAGGG

 301 ArgGlySerArgProSerTrpGlyProThrAspProArgArgArgSerArgAsnLeuGly
 CGTGGCTCTCGGCCCTAGCTGGGCCCCACAGACCCCGCGGTAGGTCGCGCAATTGGGT
 GCACCGAGAGCCGGATCGACCCCGGGGTCTCTGGGGCCGCATCCAGCGCGTTAAACCCA

 361 LysValIleAspThrLeuThrCysGlyPheAlaAspLeuMetGlyTyrIleProLeuVal
 AAGGTCATCGATACCTTACGTGGGCTTCGCCGACCTCATGGGGTACATACCGCTCGTC
 TTCCAGTAGCTATGGGAATGCACGCCGAGCGGTGGAGTACCCCATGTATGGCGAGCAG

 421 GlyAlaProLeuGlyGlyAlaAlaArgAlaLeuAlaHisGlyValArgValLeuGluAsp
 GGCGCCCTCTTGGAGCGCTGCCAGGCCCTGGCGCATGGCGTCCGGTTCTGGAAGAC
 CCGCGGGAGAACCTCCCGGACGGTCCCGGACCGCGTACCGCAGGCCCAAGACCTTCTG

FIG. 1B

FIG. 1C

481 GlyValAsnTyrAlaThrGlyAsnLeuProGlyCysSerPheSerIlePheLeuLeuAla
 GCGGTGAACATGCAACAGGGAACCTTCCTGGTGTCTTCTCTATCTTCTCTGGCC
 CCGCACTTGATACGTTGTCCCTTGGAAGGACCAACGAGAAAGAGATAGAAAGAACCCG
 Thr
 541 LeuLeuSerCysLeuThrValProAlaSerAlaTyrGlnValArgAsnSerThrGlyLeu
 CTGCTCTCTTGCTTGACTGTGCCCGCTTCGGCCTACCAAGTGCAGAACTCCACGGGGCTT
 GACGAGAGAACGAACTGACACGGGGGAGCCGGATGGTTACGCGTTGAGGTGCCCGGAA
 601 TyrHisValThrAsnAspCysProAsnSerSerIleValTyrGluAlaAlaAspAlaIle
 TACCACGTCACCAATGATTGCCCTAACTCGAGTATTGTGTACGAGCGGCCGATGCCATC
 ATGGTGCAGTGGTTACTAACGGGATTGAGCTCATAACACATGCTCCGCCGCTACGGTAG
 661 LeuHisThrProGlyCysValProCysValArgGluGlyAsnAlaSerArgCysTrpVal
 CTGCACACTCCGGGTGCGTCCCTTGCGTTCGTGAGGGCAACGCCCTCGAGGTGTGGGTG
 GACGTGTGAGGCCCCACGCAGGGAAACGCAAGCACTCCCGTTGCGGAGCTCCACAACCCAC
 721 AlaMetThrProThrValAlaThrArgAspGlyLysLeuProAlaThrGlnLeuArgArg
 GCGATGACCCCTACGGTGGCCACCAGGATGGCAAACTCCCCGCGACGACGCTTCGACGT
 CGCTACTGGGGATGCCACCGGTGTCCTACCGTTTGAGGGCGCTGCGTCAAGCTGCA
 781 HisIleAspLeuLeuValGlySerAlaThrLeuCysSerAlaLeuTyrValGlyAspLeu
 CACATCGATCTGCTTGTCGGAGCGCCACCTCTGTTCGGCCCTCTACGTGGGGACCTA
 GTGTAGCTAGACGAACAGCCCTCGCGGTGGGAGACAAGCCGGGAGATGACCCCTGGAT
 841 CysGlySerValPheLeuValGlyGlnLeuPheThrPheSerProArgArgHisTrpThr
 TGGGGTCTGCTTTCTTGTCGGCCAACTGTTACCTTCTCTCCAGCGGCCACTGGACG
 ACGCCCCAGACAGAAAGAACAGCCGGTTGACAAGTGGAAGAGAGGGTCCCGGTGACCTGC

901 ThrGlnGlyCysAsnCysSerIleTyrProGlyHisIleThrGlyHisArgMetAlaTrp
 ACGCAAGGTTGCAATTGCTCTATCTATCCCGGCCATATAACGGGTACCGCATGGCATGG
 TCGCTTCCAAACGTTAACGAGATAGATAGGGCCGGTATATTGCCAGTGCGGTACCGTACC

961 AspMetMetMetAsnTrpSerProThrThrAlaLeuValMetAlaGlnLeuLeuArgIle
 GATATGATGATGAACCTGGTCCCCTACGACGGCGTTGGTAATGGCTCAGCTGCTCCGGATC
 CTATACTACTTGACCAAGGGGATGCTGCCGCAACCATTACCGAGTCGACGAGGCCCTAG

1021 ProGlnAlaIleLeuAspMetIleAlaGlyAlaHisTrpGlyValLeuAlaGlyIleAla
 CCACAAGCCATCTTGGACATGATCGCTGGTGCTCACTGGGAGTCCTGGCGGCATAGCG
 GGTGTTCCGGTAGAACCTGTACTAGCGACCAAGTAGACCCCTCAGGACCGCCCGTATCGC

1081 TyrPheSerMetValGlyAsnTrpAlaLysValLeuValValLeuLeuPheAlaGly
 TATTTCTCCATGGTGGGAACTGGGCGAAGTCCCTGGTAGTGTGCTGTCTATTGCCGGC
 ATAAAGAGGTACCAACCCCTTGACCCGCTTCCAGGACCATCACGACGACGATAAACGGCCG

1141 ValAspAlaGluThrHisValThrGlyGlySerAlaGlyHisThrValSerGlyPheVal
 GTCGACGGGAAACCCACGTCAACGGGGGAAGTGGCGGCCACACTGTGTCTGGATTGTGT
 CAGCTGCGCCCTTTGGGTGCAGTGGCCCCCTTCAAGGCCGGTGTGACACAGACCTAAACAA

1201 SerLeuLeuAlaProGlyAlaLysGlnAsnValGlnLeuIleAsnThrAsnGlySerTrp
 AGCCTCCTCGCACCGCGCCCAAGCAGAACGTCCAGCTCATCAACACCAACGGCAGTTGG
 TCGGAGGAGCGTGGTCCGCGGTTCTGTCAGGTCCGACTAGTGTGGTTGCCGTCAACC

FIG. 1D

FIG. 1E

1261 HisLeuAsnSerThrAlaLeuAsnCysAsnAspSerLeuAsnThrGlyTrpLeuAlaGly
 CACCTCAATAGCACGGCCCTGAACCTGCAATGATAGCTCAACACCGGCTGGTTGGCAGGG
 GTGGAGTTATCGTGCCGGGACTTGACGTTACTATCGGAGTTGTGGCCGACCAACCGTCCC

 1321 LeuPheTyrHisHisLysPheAsnSerSerGlyCysProGluArgLeuAlaSerCysArg
 CTTTTCTATCACCAAGTTCAACTCTTCAGGCTGTCTGAGAGGCTAGCCAGCTGCCGA
 GAAAGATAGTGGTGTTCAGTTGAGAAAGTCCGACAGGACTCTCCGATCGGTCCGACGGCT

 1381 ProLeuThrAspPheAspGlnGlyTrpGlyProIleSerTyrAlaAsnGlySerGlyPro
 CCCCTTACCGATTTTGACACCGGCTGGGCCCTATCAGTTATGCCAACGGAGCGGCCCC
 GGGGAATGGCTAAACTGGTCCCGACCCCGGATAGTCAATACGGTTGCCTTCGCCGGGG

 1441 AspGlnArgProTyrCysTrpHisTyrProProLysProCysGlyIleValProAlaLys
 GACCAGCGCCCTACTGCTGGCACTACCCCAAAACCTTGCGGTATTGTGCCCGCGAAG
 CTGGTCGGGGGATGACGACCGTGATGGGGGTTTGTGAACGCCCATAAACACGGGCGCTTC

 1501 SerValCysGlyProValTyrCysPheThrProSerProValValGlyThrThrAsp
 AGTGTGTGTGGTCCGGTATATTGCTTCACTCCAGCCCGTGGTGGTGGAAACGACCGAC
 TCACACACACCGGCCATATAACGAAGTGAGGTGCGGGCACCAACCTTGCTGGCTG

 1561 ArgSerGlyAlaProThrTyrSerTrpGlyGluAsnAspThrAspValPheValLeuAsn
 AGGTCGGGCGCGCCACCTACAGCTGGGGTGAAATGATACGGACGCTCTTCGTCCTTAAC
 TCCAGCCCGCGGGTGATGTCGACCCCACTTTTACTATGCTGCAGAACGAGGAATTG

 1621 AsnThrArgProProLeuGlyAsnTrpPheGlyCysThrTrpMetAsnSerThrGlyPhe
 AATACCGGCCACCGCTGGGCAATTGGTTGCTGTGACCTGGATGAACCTCAACTGGATTCT
 TTATGGTCCGGTGGCGACCCGTTAACCAAGCCAACATGGACCTACTTGAGTTGACCTAAG

1681 ThrLysValCysGlyAlaProProCysValIleGlyGlyAlaGlyAsnAsnThrLeuHis
 ACCAAAGTGTGCGAGCGCTCTTGTGTATCGGAGGGGGGCAACAAACCCCTGCAC
 TGGTTTCACACGCCCTCGCGGAGGAACACAGTAGCCTCCCGCCCGTTGTGTGGACGTG

 1741 CysProThrAspCysPheArgLysHisProAspAlaThrTyrSerArgCysGlySerGly
 TGCCCCACTGATTGCTTCCGCAAGCATCCGGACGCCACATACTCTCGGTGGCTCCGGT
 ACGGGGTGACTAACGAAGCGTTCTGTAGCGCTGGGTGTATGAGAGCCACGCCGAGGCCA

 Ile
 1801 ProTrpLeuThrProArgCysLeuValAspTyrProTyrArgLeuTrpHisTyrProCys
 CCTGGATCACACCCAGGTGCTGGTCCGACTACCCGTATAGGCTTTGGCATATCCTTGT
 GGGACCTAGTGTGGTCCACGGACCGACTGATGGGCATATCCGAAACCCGTAATAGGAACA

 1861 ThrIleAsnTyrThrIlePheLysIleArgMetTyrValGlyGlyValGluHisArgLeu
 ACCATCAACTACACCATAATTAAATCAGGATGTACGTGGAGGGGTGGAACACACAGGCTG
 TGGTAGTTGATGTGGTATAAAATTTAGTCTACATGCACCTCCCGAGCTTGTGTCCGAC

 1921 GluAlaAlaCysAsnTrpThrArgGlyGluArgCysAspLeuGluAspArgAspArgSer
 GAAGCTGCCCTGCAACTGGACGGGGGCGAACGTTGCGATCTGGAAGACAGGACAGGTCC
 CTTCCAGCGGACGTTGACCTGCGCCCCCGCTTGCAACGCTAGACCTTCTGTCCCTGTCCAGG

 1981 GluLeuSerProLeuLeuLeuThrThrThrGlnTrpGlnValLeuProCysSerPheThr
 GAGCTCAGCCCGTTACTGCTGACCACTACACAGTGGCAGGTCTCCCGTGTTCCTTCACA
 CTCGAGTCGGGCAATGACGACTGGTGATGTGTCAACCGTCCAGGAGGGGCACAAGGAAGTGT

FIG. 1F

FIG. 1G

2041 ThrLeuProAlaLeuSerThrGlyLeuIleHisLeuHisGlnAsnIleValAspValGln
 ACCCTACCAGCCTTGTCCACCGGCTCATCCACCTCCACCAGAACATTGTGGACGTGCAG
 TGGGATGGTCGGAACAGGTGCGCGGAGTAGGTGGAGGTGCTTGTAAACACCTGCACGTC

 2101 TyrLeuTyrGlyValGlySerSerIleAlaSerTrpAlaIleLysTrpGluTyrValVal
 TACTTGTAACGGGTGGGTCAAGCATCGCGTCTCTGGGCCATTAAAGTGGAGTACGTCGTT
 ATGAACATGCCCCACCCAGTTCTGTAGCGCAGGACCCGGTAATTCAACCCTCATGCAGCAA

 2161 LeuLeuPheLeuLeuAlaAspAlaArgValCysSerCysLeuTrpMetMetLeuLeu
 CTCCTGTTCCTTCTGCTTGACAGACGCGCGCTCTGCTCCTGCTGTGGATGATGCTACTC
 GAGGACAAAGGAAGACGAACGCTCTGCGCGCAGACGAGGACGAACACCTACTACGATGAG

 2221 IleSerGlnAlaGluAlaLeuGluAsnLeuValIleLeuAsnAlaAlaSerLeuAla
 ATATCCCAAGCGGAGCGGCTTTGGAGAACCTCGTAATACTTAATGCAGCATCCCTGGCC
 TATAGGGTTGCGCTCCGCCGAAACCTCTTGGAGCATTAATGAATTACGTCGTAGGGACCGG

 2281 GlyThrHisGlyLeuValSerPheLeuValPhePheCysPheAlaTrpTyrLeuLysGly
 GGGACGCACGGTCTTGATCCTTCCTCGTGTCTTCTGCTTTGCATGGTATTGAAGGT
 CCTGCGTGCCAGAACATAGGAAGGAGCACAAAGACGAAACGTACCATAACTTCCCA

 2341 LysTrpValProGlyAlaValTyrThrPheTyrGlyMetTrpProLeuLeuLeuLeu
 AAGTGGTGCCCGGAGCGGTCTACACCTTCTACGGGATGTGGCCTCTCCTCCTCCTG
 TTCACCCACGGGCTCGCCAGATGTGGAGATGCCCTACACCGGAGAGGACGAGGAC

 2401 LeuAlaLeuProGlnArgAlaTyrAlaLeuAspThrGluValAlaAlaSerCysGlyGly
 TTGGCGTTGCCCGGCGGTACCGGCTGGACACGAGGTGGCCGCGTCTGTGGCGGT
 AACCGCAACGGGGTCCGCCGATGCGGACCTGTGCTCCACCGCGCAGCACACCGCCA

2461 ValValLeuValGlyLeuMetAlaLeuThrLeuSerProTyrTyrLysArgTyrIleSer
 GTTGTCTCGTCGGGTTGATGGCGTGACTCTGTCAACCATATTACAAGCGCTATATCAGC
 CAACAAGAGCAGCCCCAACTACCGCGACTGAGACAGTGGTATAATGTTGCGGATATAGTCG

 (Asn)
 2521 TrpCysLeuTrpTrpLeuGlnTyrPheLeuThrArgValGluAlaGlnLeuHisValTrp
 TGGTGGCTTGTGGCTTCAGTATTTTCTGACCAAGAGTGGAGCGCAACTGCACGTGTGG
 ACCACGAACACACCCGAAAGTCATAAAAGACTGGTCTCACCTTCGCGTTGACGTGCACACC

 2581 IleProLeuAsnValArgGlyGlyArgAspAlaValIleLeuLeuMetCysAlaVal
 ATTCCCCCCCCCTCAACGTCGAGGGGGCGGACCGTCATCTTACTCATGTGTGCTGTA
 TAAGGGGGGAGTTGCAGGCTCCCCCGCTGCGGCAGTAGAATGATACACACGACAT

 2641 HisProThrLeuValPheAspIleThrLysLeuLeuAlaValPheGlyProLeuTrp
 CACCCGACTCTGGTATTGACATCACCAAAATTGCTGCTGGCGTCTTCGGACCCCTTGG
 GTGGGCTGAGACCATAAACTGTAGTGGTTTAAACGACGACCGGAGAAAGCTGGGGAAACC

 2701 IleLeuGlnAlaSerLeuLeuLysValProTyrPheValArgValGlnGlyLeuLeuArg
 ATTCTTCAAGCCAGTTTGCTTAAAGTACCTACTTTGTGCGCGTCCAAGGCCCTTCTCCGG
 TAAGAAGTTCGGTCAAAACGAATTTTCATGGGATGAAACACGCGCAGGTTCCGGAGAGGCC

 2761 PheCysAlaLeuAlaArgLysMetIleGlyGlyHisTyrValGlnMetValIleIleLys
 TTCTGCGCGTTAGCGCGGAAGATGATCGGAGGCCATTACGTGCAAAATGGTCATCATTAAG
 AAGACGGCAATCGCGCCTTCTACTAGCCTCCGGTAATGCACGTTTACCAGTAGTAATTC

FIG. 1H

FIG. 11

2821	LeuGlyAlaLeuThrGlyThrTyValTyAsnHisLeuThrProLeuArgAspTrpAla TTAGGGCGCTTACTGGACCTATGTTTATAAACCATCTCACTCCTCTTCGGGACTGGGCG AATCCCCGGAATGACCGTGGATACAAATATTGGTAGAGTGAGGAGAACCCCTGACCCGC
2881	HisAsnGlyLeuArgAspLeuAlaValGluProValValPheSerGlnMetGlu CACAAACGGCTTGCGAGATCTGGCCGTGGCTGTAGAGCCAGTCGTCCTCTCCCAAATGGAG GTGTTGCCGAACGCTCTAGACCGGCACCGACATCTCGGTCAAGAGAGGTTTACCTC
2941	ThrLysLeuIleThrTrpGlyAlaAspThrAlaAlaCysGlyAspIleIleAsnGlyLeu ACCAAGCTCATCACGTGGGGGAGATACCGCCCGTGGGTGACATCATCAACGGCTTG TGGTTCGAGTAGTGACCCCCCGTCTATGGCGGCGCACGCCACTGTAGTAGTTGCCGAAC
3001	ProValSerAlaArgArgGlyArgGluIleLeuLeuGlyProAlaAspGlyMetValSer CCTGTTTCCGCCCGAGGGCGGGAGATAGTCTCGGGCCAGCCGATGGAATGCTCTCC GGACAAAGCGGGCGTCCCCGGCCCTCTATGACGAGCCCGGTCCGCTACCTTACCAGAGG
3061	LysGlyTrpArgLeuLeuAlaProIleThrAlaTyrAlaGlnGlnThrArgGlyLeuLeu AAGGGTGAGGTTGCTGGCGCCCATCACGGCGTAGTACGCCACGACAGCAAGGGCCCTCCTA TTCCCCACCTCCAACGACCGGGTAGTCCCGCATGCGGGTCTGTCTCCCCCGGAGGAT
3121	GlyCysIleIleThrSerLeuThrGlyArgAspLysAsnGlnValGluGlyGluValGln GGTGCCATAATCACAGCCTAACTGGCCGGGACAAACCAAGTGAGGTGAGGTCCAG CCCACGTATTAGTGGTCGGATTGACCGGCCCTGTTTGTGTTCACTCCCACTCCAGGTC
3181	IleValSerThrAlaAlaGlnThrPheLeuAlaThrCysIleAsnGlyValCysTrpThr ATTGTGTCAACTGCTGCCCAAACCTTCCTGGCAACGTGCATCAATGGGGTGTGCTGGACT TAACACAGTTGACGACGGGTTTGGAAGGACCGTTGCACGTAGTTACCCCAACGACCTGA

3241 ValTyrHisGlyAlaGlyThrArgThrIleAlaSerProLysGlyProValIleGlnMet
 GTCTACCAAGGGCCGGAACGAGGACCATCGCGTCACCCAAAGGTCCTGTCTATCCAGATG
 CAGATGGTGCCCGCCCTTGCTCCTGGTAGCGCAGTGGTTCCTCCAGGACAGTAGGTCTAC

3301 TyrThrAsnValAspGlnAspLeuValGlyTrpProAlaProGlnGlySerArgSerLeu
 TATACCAATGTAGACCAAGACCTTGTTGGGCTGGCCCCGTCCGCAAGTAGCCGCTCATTTG
 ATATGGTTACATCTGGTTCTGGAAACACCCGACCGGGCGAGCGTTCATCGGCGAGTAAC

3361 ThrProCysThrCysGlySerSerAspLeuTyrLeuValThrArgHisAlaAspValIle
 ACACCTGCACCTGCGGCTCCTCGGACCTTTACCTGGTCACGAGGCACGCCGATGTCAATT
 TGTGGGACGTGAACGCCGAGGAGCCTGGAAATGGACCAAGTCTCCGTCCGGCTACAGTAA

3421 ProValArgArgGlyAspSerArgGlySerLeuLeuSerProArgProIleSerTyr
 CCGTGCGCGCGGGGTGATAGCAGGGGAGCCCTGCTGTCGCCCCGCCCATTTCTCTAC
 GGGCACGCGCGCCCTACTATCGTCCCGTCGGACGACAGCGGGGCGGGTAAAGGATG

3481 LeuLysGlySerSerGlyGlyProLeuLeuCysProAlaGlyHisAlaValGlyIlePhe
 TTGAAAGGCTCCTCGGGGGTCCGCTGTGTGTGCCCCCGGGGCACGCCGTGGCATATTT
 AACTTCCGAGGAGCCCCCAGGCGACACACGCGGGCGCCCCGTGCGGCACCCGTATAAA

3541 ArgAlaAlaValCysThrArgGlyValAlaLysAlaValAspPheIleProValGluAsn
 AGGCCCGCGGTGTGCACCCGTGGAGTGGCTAAGCGGTGGACTTTATCCCTGTGGAGAAC
 TCCCGGCGCCACACGTGGGACCTCACCGATTCCGCCACCTGAAATAGGGACACCTCTTG

FIG. 1J

FIG. 1K

3601 LeuGluThrThrMetArgSerProValPheThrAspAsnSerSerProProValValPro
 CTAGAGACAACCATGAGGTCCCGGTGTTACGGATAAATCTCTCTCCACCATGAGTGCCT
 GATCTCTGTTGGTACTCCAGGGGCCACAAGTGCCTATTGAGGAGAGGTGTCATCACGGG

 3661 GlnSerPheGlnValAlaHisLeuHisAlaProThrGlySerGlyLysSerThrLysVal
 CAGAGCTTCCAGGTGGCTCACCTCCATGCTCCACAGGCAGCGGCAAAAGCACCAAGGTC
 GTCTCGAAGGTCCACCGAGTGGAGGTACGAGGGTCCGTCCGTCTTTCGTGTTCCAG

 3721 ProAlaAlaTyrAlaAlaGlnGlyTyrLysValLeuValLeuAsnProSerValAlaAla
 CCGGCTGCATATGCAGCTCAGGGCTATAAGGTGCTAGTACTCAACCCCTCTGTGCTGCA
 GGCCGACGTATACGTCGAGTCCCGATATATCCACCATCATGAGTTGGGGAGACACGACGT

 3781 ThrLeuGlyPheGlyAlaTyrMetSerLysAlaHisGlyIleAspProAsnIleArgThr
 ACATGGGCTTTGGTGCTTACATGTCCAAGGCTCATGGGATCGATCCTAACATCAGGACC
 TGTGACCCCGAAACCAACGAATGTACAGGTTCCGAGTACCCCTAGCTAGGATTGTAGTCTGG

 3841 GlyValArgThrIleThrThrGlySerProIleThrTyrSerThrTyrGlyLysPheLeu
 GGGGTGAGAACAAATTACCACTGGCAGCCCCCATCACGTACTCCACCTACGGCAAGTTCCTT
 CCCCACCTCTTGTAAATGGTGACCGTCGGGGTAGTGCATGAGGTGGATGCCGTTCAAGGAA

 3901 AlaAspGlyGlyCysSerGlyGlyAlaTyrAspIleIleCysAspGluCysHisSer
 GCCGACGGCGGTGCTCGGGGGCGCTTATGACATAATAATTTGTGACGAGTGCCACTCC
 CGGCTGCCCGCCACGAGCCCCCGGGAATACTGTATTATTAAACACTGCTCACGGTGAGG

Leu

3961 ThrAspAlaThrSerIleLeuGlyIleGlyThrValLeuAspGlnAlaGluThrAlaGly
 ACGGATGCCACATCCATCTTGGGCATCGGCACCTGCTCCTTGACCAAGCAGAGACTGCGGG
 TGCCTACGGTGTAGGTAGAACCCGTAGCCGTGACAGGAACCTGGTTCGTCTCTGACGCCCC
 (Val)
 4021 AlaArgLeuValValLeuAlaThrAlaThrProProGlySerValThrValProHisPro
 GCGAGACTGGTGTGCTCGCCACCGCCACCCCTCCGGCTCCGTCACCTGTGCCCATCCC
 CGCTCTGACCAACACGAGCGGTGGGTGGGAGGCCCGAGGCAGTGACACGGGGTAGGG
 4081 AsnIleGluGluValAlaLeuSerThrThrGlyGluIleProPheTyrGlyLysAlaIle
 AACATCGAGAGGTTGCTCTGTCCACCACCGGAGAGATCCCTTTTACGGCAAGGCTATC
 TTGTAGCTCCTCCAACGAGACAGGTGGTGGCTCTCTAGGGAATAATGCCGTTCGGATAG
 4141 ProLeuGluValIleLysGlyGlyArgHisLeuIlePheCysHisSerLysLysCys
 CCCCTCGAAGTAATCAAGGGGGGAGACATCTCATCTTCTGTCTCATTCAAAGAAGATGC
 GGGAGCTTCATTAGTTCCCCCTCTGTAGTAGTAGAAGACAGTAAGTTCTTCTTCACG
 4201 AspGluLeuAlaAlaLysLeuValAlaLeuGlyIleAsnAlaValAlaTyrTyrArgGly
 GACGAACTCGCCGCAAGCTGGTCGCATTTGGGCATCAATGCCGTGGCCTACTACCGCGGT
 CTGCTTGAGCGCGTTTCGACCCAGCGTAACCCGTAGTTACGGCACCGGATGATGGCGCCA
 4261 LeuAspValSerValIleProThrSerGlyAspValValValAlaThrAspAlaLeu
 CTTGACGTGTCCGTCAATCCGACCGCGGATGTTGTCTCGTGGCAACCGATGCCCTC
 GAACTGCACAGGCAGTAGGGCTGGTCCCGCTACAACAGCAGCACCGTTGGCTACGGGAG
 4321 MetThrGlyTyrThrGlyAspPheAspSerValIleAspCysAsnThrCysValThrGln
 ATGACCGGTATACCGGCGACTTCGACTCGGTGATAGACTGCAATACGTGTGTACCCAG
 TACTGGCCGATATGGCCGCTGAAGCTGAGCCACTATCTGACGTTATGCACACAGTGGGTC
 Tyr

FIG. 1L

FIG. 1M

(Ser)

ThrValAspPheSerLeuAspProThrPheThrIleGluThrIleThrLeuProGlnAsp
 4381 ACAGTCGATTTCAGCCTTGACCTACCTTACCAATTGAGACAATCAGCTCCCCCAGGAT
 TGTCAGCTAAAGTCGGAACCTGGGATGGAAGTGGTAACTCTGTAGTGGAGGGGTCTTA

 AlaValSerArgThrGlnArgArgGlyArgThrGlyArgGlyLysProGlyIleTyrArg
 4441 GCTGTCTCCCGCACTCAACGTCGGGCGAGGACTGGCAGGGGAAGCCAGGCATCTACAGA
 CGACAGAGGGCGTGAGTTGCAGCCCCGTCCTGACCGTCCCCCTTCGGTCCGTAGATGTCT

 PheValAlaProGlyGluArgProSerGlyMetPheAspSerSerValLeuCysGluCys
 4501 TTTGTGGCACCGGGAGCGCCCCCTCCGGCATGTTCTGACTCGTCCGTCCTCTGTGAGTGC
 AAACACCGTGGCCCCCTCGCGGGGAGGCCGTACAAGCTGAGCAGGCGAGGACACTCAGC

 TyrAspAlaGlyCysAlaTrpTyrGluLeuThrProAlaGluThrThrValArgLeuArg
 4561 TATGACGCAGGCTGTGCTTGGTATGAGCTACGCCCGCCGAGACTACAGTTAGGCTACGA
 ATACTGCGTCCGACACGAAACCATACTCGAGTGGCGGCTCTGTATGTCAATCCGATGCT

 AlaTyrMetAsnThrProGlyLeuProValCysGlnAspHisLeuGluPheTrpGluGly
 4621 GCGTACATGAACACCCCGGGCTTCCCGTGTGCCAGGACCATCTTGAATTTGGGAGGGC
 CGCATGTACTTGTGGGCCCCCGAAGGGCACACGGTCTCTGTAGAACTTAAACCCCTCCCG

 ValPheThrGlyLeuThrHisIleAspAlaHisPheLeuSerGlnThrLysGlnSerGly
 4681 GTCTTTACAGGCCTCACTCATATAGATGCCCACTTTCTATCCAGACAAAGCAGAGTGGG
 CAGAAATGTCCGGAGTGAGTATATCTACGGGTGAAAGATAGGTCTGTTCGTCTCACCC

 GluAsnLeuProTyrLeuValAlaTyrGlnAlaThrValCysAlaArgAlaGlnAlaPro
 4741 GAGAACCTTCCCTTACCTGGTAGCGTACCAAGCCACCGTGTGCGCTAGGCTCAAGCCCCCT
 CTCTTGGAAGGAATGGACCATCGCATGGTTCGGTGGCACACGGGATCCCGAGTTCGGGGA

4801 ProProSerTrpAspGlnMetTrpLysCysLeuIleArgLeuLysProThrLeuHisGly
 CCCCATCGTGGACCATGATGTGAAAGTGTGATTCGCCTCAAGCCACCCCTCCATGGG
 GGGGTAGCACCCCTGGTCTACACCTTCACAACTAAGCGGAGTTCGGGTGGAGGTACCC

 4861 ProThrProLeuLeuTyArgLeuGlyAlaValGlnAsnGluIleThrLeuThrHisPro
 CCAACACCCCTGCTATACAGACTGGGCGCTGTTCAGAAATGAAATCACCCCTGACGCCCA
 GGTGTGGGACGATATGTCTGACCCGCGACAAGTCTTACTTAGTGGGACTGCGTGGGT

 4921 ValThrLysTyrlleMetThrCysMetSerAlaAspLeuGluValValThrSerThrTrp
 GTCACCAATAACATCATGACATGTCATGTGCGCCGACCTGGAGGTCTGTCACGACACCTGG
 CAGTGGTTTATGTAGTACTGTACGTACAGCCGGCTGGACCTCCAGCAGTGTCTCGTGACC

 4981 ValLeuValGlyGlyValLeuAlaAlaLeuAlaAlaTyrcysLeuSerThrGlyCysVal
 GTGCTCGTTGGCGGCTCCTGGCTGCTTTGGCCGCGTATTGCCCTGTCAACAGGCTGCGTG
 CACGAGCAACCCGCCAGGACCGAGAAACCGCGGCATACGGACAGTTGTCCGACGCAC

 5041 ValIleValGlyArgValValLeuSerGlyLysProAlaIleIleProAspArgGluVal
 GTCATAGTGGCAGGTCGTCTTGTCCGGAAAGCCGGCAATCATACCTGACAGGGAAGTC
 CAGTATCACCCGTCCTCCAGCAGAACAGGCCCTTCGGCCGTTAGTATGGACTGTCCCTTCAG

 5101 LeuTyArgGluPheAspGluMetGluGluCysSerGlnHisLeuProTyrlleGluGln
 CTCTACCGAGAGTTCGATGAGATGGAAGAGTGTCTCTCAGCACTTACCGTACATCGAGCAA
 GAGATGGCTCTCAAGCTACTCTACCTTCTCAGCAGAGTCGTGAATGGCATGTAGCTCGTT

FIG. 1N

FIG. 10

5161	GlyMetMetLeuAlaGluGlnPheLysGlnLysAlaLeuGlyLeuLeuGlnThrAlaSer GGGATGATGCTCGCCGAGCAGTTCAAGCAGAAGGCCCTCGGCCCTCCTGCAGACCGCGTCC CCCTACTACGAGCGGCTCGTCAAGTTCGTCTCCGGGAGCCGGAGGACGTCTGGCGCAGG
5221	ArgGlnAlaGluValIleAlaProAlaValGlnThrAsnTrpGlnLysLeuGluThrPhe CGTCAGGCAGAGGTTATCGCCCTCTGTGTCCAGACCAACTGGCAAAACTCGAGACCTTC GCAGTCCGTCTCCAATAGCGGGACGACAGGCTCTGGTTGACCGTTTTTGTAGCTCTGGAAG
5281	TrpAlaLySHisMetTrpAsnPheIleSerGlyIleGlnTyrLeuAlaGlyLeuSerThr TGGGCGAAGCATATGTGGAACCTTCATCAGTGGGATACAATACTTGGCGGCTTGTC AACG ACCCGCTTCGTATACACCTTGAAGTAGTCACCCCTATGTTATGAACCGCCCGAACAGTTGC
5341	LeuProGlyAsnProAlaIleAlaSerLeuMetAlaPheThrAlaAlaValThrSerPro CTGCCCTGGTAACCCGCCATTGCTTCATTGATGGCTTTTACAGCTGCTGTCAACGCCCCA GACGGACCATTGGGGCGGTAACGAAGTAACTACCGAAATGTCTGACGACAGTGGTCCGGT
5401	LeuThrThrSerGlnThrLeuLeuPheAsnIleLeuGlyGlyTrpValAlaAlaGlnLeu CTAACCACTAGCCAAACCCCTCCTCTTCAACATATTGGGGGGTGGTGGCTGCCAGCTC GATTGGTGATCGGTTTGGGAGGAGAGTTGTATAACCCCCCAACCGACGGGTCCGAG
5461	AlaAlaProGlyAlaAlaThrAlaPheValGlyAlaGlyLeuAlaGlyAlaAlaIleGly GCCGCCCGGTGCCGCTACTGCCCTTGTGGCGCTGGCTTAGCTGGCGCCGCTATCGGC CGCGGGGCCACGGCGATGACGGAAACACCCGCGACCGAATCGACCGCGGTAGCCG
5521	SerValGlyLeuGlyLysValLeuIleAspIleLeuAlaGlyTyrGlyAlaGlyValAla AGTGTGGACTGGGAAGGTCCCTCATAGACATCCTTGCAGGGTATGGCGCGCGGTGGCG TCACAACCTGACCCCTTCCAGGAGTATCTGTAGGAACGTCCCATACCGCGCCGACCCG

5581 GlyAlaLeuValAlaPheLysIleMetSerGlyGluValProSerThrGluAspLeuVal
 (Gly)
 GGAGCTTTGTGGCATTCAGATCATGAGCGGTGAGGTCCCTCCACGGAGGACCTGGTC
 CCTCGAGAACACCGTAAGTTCTAGTACTCGCCACTCCAGGGGAGGTGCTCTCTGGACCCAG

 5641 AsnLeuLeuProAlaIleLeuSerProGlyAlaLeuValValGlyValValCysAlaAla
 AATCTACTGCCCGCCATCCTCTCGCCCGAGCCCTCGTAGTCGGCGTGTCTGTGCAGCA
 TTAGATGACGGCGGTAGGAGAGCGGGCCTCGGGAGCATCAGCCGCCACGACACGTCGT

 5701 IleLeuArgArgHisValGlyProGlyGluGlyAlaValGlnTrpMetAsnArgLeuIle
 ATACTGCCCGCCGACGTTGGCCCGGAGGGGAGTGCAGTGGATGAACCGGCTGATA
 TATGACGGCGCGGTGCAACCGGGCCGCTCCCCGTACGTCACTTGGCCGACTAT

 5761 AlaPheAlaSerArgGlyAsnHisValSerProThrHisTyrValProGluSerAspAla
 GCCTTCGCCCTCCCGGGGAACCATGTTTCCCCACGCACACTACGTGCCGGAGCGCATGCA
 CGGAAGCGGAGGGCCCCCTTGGTACAAAGGGGGTGGGTGATGCACGGCCCTCTCGCTACGT

 5821 (HisCys)
 AlaAlaArgValThrAlaIleLeuSerSerLeuThrValThrGlnLeuLeuArgArgLeu
 GCTGCCCGCGTCACTGCCATACTCAGCAGCCCTCACTGTAAACCCAGCTCCTGAGGCGACTG
 CGACGGGCGCAGTGACGGTATGAGTCGTGGAGTGACATTTGGGTGCGAGGACTCCGCTGAC

 5881 HisGlnTrpIleSerSerGluCysThrThrProCysSerGlySerTrpLeuArgAspIle
 CACCAAGTGGATAAGCTCGGAGTGTAACCACTCCATGCTCCGGTCTCTGGCTAAGGGACATC
 GTGGTCACCTATTTCGAGCCCTCACATGGTGAGGTACGAGGCCAAGGACCGATTCCCTGTAG

FIG. 1P

FIG. 1Q

5941 TrpAspTrpIleCysGluValLeuSerAspPheLysThrTrpLeuLysAlaLysLeuMet
 TGGGACTGGATATGCGAGGTGTGAGCGACTTTAAGACCTGGCTAAAGCTAAGCTCATG
 ACCCTGACCTATACGCTCCACAACACTCGCTGAAATTCTGGACCGATTTCGATTGAGTAC

 6001 ProGlnLeuProGlyIleProPheValSerCysGlnArgGlyTyrLysGlyValTrpArg
 CCACAGCTGCCCTGGGATCCCCCTTTGTGTCTCTGCCAGCGGGTATAAGGGGTCTGGCGA
 GGTGTCGACGGACCCCTAGGGGAAACACAGGACGGTCCGGCCCATATATCCCCAGACCGCT
 (Val)
 6061 GlyAspGlyIleMethIleThrArgCysHisCysGlyAlaGluIleThrGlyHisValLys
 GTGGACGGCATCATGCACACTCGCTGCCACTGTGGAGCTGAGATCACTGGACATGTCAAA
 CACCTGCCGTAGTACGTGTGAGCGACGGTGACACCTCGACTCTAGTGACCTGTACAGTTT

 6121 AsnGlyThrMetArgIleValGlyProArgThrCysArgAsnMetTrpSerGlyThrPhe
 AACGGACGATGAGGATCGTCGGTCCTAGGACCTGCAGGAACATGTGGAGTGGACCTTC
 TTGCCCTGCTACTCCTAGCAGCCAGGATCCTGGACGTCCTTGATACACCTCACCCCTGGAAAG

 6181 ProIleAsnAlaTyrThrThrGlyProCysThrProLeuProAlaProAsnTyrThrPhe
 CCCATTAAATGCCCTACACACGGGCCCTGTACCCCTTCTCGGCCGAACACTACACGTTTC
 GGTAAATTACGGATGTGGTGCCCGGGACATGGGGGAAGGACGCGGCTTGATGTGCAAG

 6241 AlaLeuTrpArgValSerAlaGluGluTyrValGluIleArgGlnValGlyAspPheHis
 GCGCTATGGAGGGTGTCTGCAGAGGAATATGTGGAGATAAGGCAGGTGGGACTTCCAC
 CGCGATACCTCCACAGACGTCTCTTATATACACCTCTATTCCGTCCACCCCTGAAGGTG

 6301 TyrValThrGlyMetThrThrAspAsnLeuLysCysProCysGlnValProSerProGlu
 TACGTGACGGGTATGACTACTGACAAATCTCAAAATGCCCGTGCCAGGTCCCATCGCCCGAA
 ATGCACTGCCCATACTGATGACTGTAGAGTTTACGGGACCGGTCCAGGGTAGCGGGCTT

6361 PhePheThrGluLeuAspGlyValArgLeuHisArgPheAlaProProCysLysProLeu
 TTTTTCACAGAAATTGGACGGGTGCGCCTACATAGGTTTGGCCCCCCTGCAAGCCCTTG
 AAAAAGTGCTTAACCTGCCCCACGCGGATGTATCCAAACGCGGGGACGTTTCGGGAAC

 6421 LeuArgGluGluValSerPheArgValGlyLeuHisGluTyrProValGlySerGlnLeu
 CTGCGGAGGAGGTATCATTCAGAGTAGGACTCCACGAATACCCGGTAGGTCGCAATTA
 GACGCCCTCCTCATAGTAAGTCTCATCTGAGGTGCTTATGGGCCATCCCAGCGTTAAT

 6481 ProCysGluProGluProAspValAlaValLeuThrSerMetLeuThrAspProSerHis
 CCTTGGAGCCCCGAACCGACGTGGCCGTGTGACGTCCATGCTCACTGATCCCTCCCAT
 GGAAACGCTCGGGCTTGGCCCTGCACCGGCACAACTGCAGGTACGAGTACTAGGGAGGTA

 6541 IleThrAlaGluAlaAlaGlyArgArgLeuAlaArgGlySerProProSerValAlaSer
 ATAAACAGCAGAGCGCGCGCGGAAGTTGGCGAGGGATCACCCCCCTCTGTGGCCAGC
 TATTGTCTCTCCGCGCGCGCTTCCAAACCGCTCCCCCTAGTGGGGGAGACACCGGTCTG

 6601 SerSerAlaSerGlnLeuSerAlaProSerLeuLysAlaThrCysThrAlaAsnHisAsp
 TCCTCGGCTAGCCAGCTATCCGCTCCATCTCTCAAGGCAACTTGACCCGCTAACCATGAC
 AGGAGCCGATCGGTCGATAGGCGAGGTAGAGAGTTCCGTTGAACGTGGCGATTGGTACTG

 6661 SerProAspAlaGluLeuIleGluAlaAsnLeuLeuTrpArgGlnGluMetGlyGlyAsn
 TCCCCGTGATGCTGAGCTCATAGAGGCCAACCTCCTATGGAGGCAGGAGATGGCGGCAAC
 AGGGGACTACGACTCGAGTATCTCCGGTTGGAGGATACCTCCGCTCCTACCCCGCGTTG

FIG. 1R

FIG. 1S

IleThrArgValGluSerGluAsnLysValValIleLeuAspSerPheAspProLeuVal
 6721 ATCACCCAGGGTTGAGTCAGAAAACAAAGTGGTATTCTGGACTCCTTCGATCCCGCTTG
 TAGTGGTCCCAACTCAGTCTTTTGTTCACCACTAAGACCTGAGGAAGCTAGCGCAACAC
 AlaGluGluAspGluArgGluIleSerValProAlaGluIleLeuArgLysSerArgArg
 6781 GCGGAGGAGGACGAGCGGAGATCTCCGTACCCGCAGAAATCCTGCGGAAGTCTCGGAGA
 CGCCTCCTCCTGCTCGCCCTCTAGAGGCATGGGCGTCTTTAGGACGCGCTTCAGAGCCTCT
 PheAlaGlnAlaLeuProValTrpAlaArgProAspTyrAsnProProLeuValGluThr
 6841 TTCGCCCCAGGCCCTGCCCGTTTGGCGCGCGGACTATAACCCCCCGCTAGTGGAGACG
 AAGCGGTCCGGACGGGCAACCCGCGCGCGCTGATATTGGGGGCGGATCACCTCTGC
 TrpLysLysProAspTyrGluProProValValHisGlyCysProLeuProProLys
 6901 TGGAAAAGCCCCGACTACGAACCACTGTGGTCCATGGCTGTCCGCTTCCACCTCCAAAG
 ACCTTTTTCGGGCTGATGCTTGGTGGACACCCAGGTACCGACAGCGGAAGGTGGAGTTTC
 SerProProValProProArgLysLysArgThrValValLeuThrGluSerThrLeu
 6961 TCCCCCTCCTGTGCCCTCCGCCCTCGGAAGAACGCGACGGTGGTCCCTCACTGAATCAACCCCTA
 AGGGAGGACACGGAGCGGAGCCTTCTTCGCCCTGCCACCCAGGAGTGACTTAGTTGGGAT
 (Ser)
 SerThrAlaLeuAlaGluLeuAlaThrArgSerPheGlySerSerSerThrSerGlyIle
 7021 TCTACTGCCCTTGGCCGAGCTCGCCACCAAGAGCTTTGGCAGCTCCTCAACTCCGGCATT
 AGATGACGGAAACCGGCTCGAGCGGTGCTTCGAAACCGTCGAGGAGTTGAAGGCCGTAA
 ThrGlyAspAsnThrThrThrSerSerGluProAlaProSerGlyCysProProAspSer
 7081 ACGGGCGACAATACGACAACATCCTCTGAGCCCCCCTTCTGTGGTGCCTCCCGACTCC
 TGCCCGCTGTATGCTGTTGTAGGAGACTCGGGCGGGGAAGACCGAGCGGGGCTGAGG

(PheAla)

7141 AspAlaGluSerTyrSerSerMetProProLeuGluGlyGluProGlyAspProAspLeu
 GACGCTGAGTCCTATTCCTCCATGCCCTCCCTGGAGGGAGCCCTGGGATCCGGATCTT
 CTGCCACTCAGGATAAGGAGGTACGGGGGGGACCTCCCCCTCGGACCCCTAGGCCTAGAA

7201 SerAspGlySerTrpSerThrValSerSerGluAlaAsnAlaGluAspValValCysCys
 AGCGACGGGTCAATGTCACCGTCAGTAGTGAGGCCAACGCGGAGGATGTCTGTGTGCTGC
 TCGCTGCCCCAGTACCAGTTGCCAGTCACTCCGGTTGGCCTCCTACAGCACACGACG

7261 SerMetSerTyrSerTrpThrGlyAlaLeuValThrProCysAlaAlaGluGluGlnLys
 TCAATGTCTTACTCTTGGACAGGCGCACTCGTCACCCCTGCGCGCGGAAGAACAGAAA
 AGTTACAGAAATGAGAACCTGTCCGCGTGAGCAGTGGGGCACGCGGCCCTTCTGTCTTT

7321 LeuProIleAsnAlaLeuSerAsnSerLeuLeuArgHisAsnLeuValTyrSerThr
 CTGCCCCATCAATGCACCTAAGCAACTCGTTGCTACGTCAACCAATTTGGTGTATTCACCC
 GACGGGTAGTTACGTGATTCTGTTGAGCAACGATGCAGTGGTGTAAACCATAGGTGG

7381 ThrSerArgSerAlaCysGlnArgGlnLysLysValThrPheAspArgLeuGlnValLeu
 ACCTCACGCAGTGCTTGCCAAAGGCAGAAAGTCAATTCACAGACTGCAAGTTCTG
 TGGAGTGGGTCAACGAACGGTTTCCGTCTTCTTTCAGTGTAACCTGTCTGACGTTCAAGAC

7441 AspSerHisTyrGlnAspValLeuLysGluValLysAlaAlaSerLysValLysAla
 GACAGCCATTACACGACGCTACTCAAGAGGTTAAAGCAGCGCGCTCAAAGTGAAGGCT
 CTGTCCGGTAATGGTCCCTGCATGAGTTCTCCTCAATTTTCGTCCCGCAGTTTTCACCTCCGA

FIG. 1T

FIG. 1U

7501	(Phe) AsnLeuSerValGluGluAlaCysSerLeuThrProProHisSerAlaLysSerLys AACTTGCTATCCGTAGAGGAAGCTTGACGCTGACGCCCCACACTCAGCCAAATCCAAG TTGAACGATAGGCATCTCCTTCGAACGTCGGACTGCGGGGTGTGAGTCGGTTAGGTTT
7561	PheGlyTyrGlyAlaLysAspValArgCysHisAlaArgLysAlaValThrHisIleAsn TTTGTTATGGGCAAAAGACGTCCGTTGCCATGCCAGAAAGCCGTAACCCACATCAAC AAACCAATACCCCGTTTCTGACGCAACGGTACGGTCTTTCCGGCATTTGGGTGAGTTG
7621	SerValTrpLysAspLeuLeuGluAspAsnValThrProIleAspThrThrIleMetAla TCCGTGTGAAAGACCTTCTGGAAGACAAATGTAAACCAATAGACACTACCATCATGGCT AGGCACACCTTCTGGAAGACCTTCTGTATCATTTGTGGTTATCTGTGATGGTAGTACCGA
7681	LysAsnGluValPheCysValGlnProGluLysGlyGlyArgLysProAlaArgLeuIle AAGAACGAGGTTTCTGCGTTCAGCCTGAGAAAGGGGGTTCGTAAGCCAGCTCGTCTCATC TTCTTGCTCCAAAGACGCAAGTCGGACTCTTCCCCCAGCATTCGGTCGAGCAGAGTAG
7741	ValPheProAspLeuGlyValArgValCysGluLysMetAlaLeuTyrAspValValThr GTGTTCCCGATCTGGCGGTGCGCGTGTGCGAAAGATGGCTTTGTACGACGTGGTTACA CACAAGGGGCTAGACCCGACGCGCACACGCTTTTCTACCGAAACATGTCACCAATGT
7801	LysLeuProLeuAlaValMetGlySerSerTyrGlyPheGlnTyrSerProGlyGlnArg AAGCTCCCTTGCGGTGATGGGAAGCTCCTACGGATTCCAATACTCACAGGACAGCGG TTCGAGGGGAACCGGCACTACCCCTTCGAGGATGCCTAAGTTATGAGTGGTCCCTGTCGCC
7861	ValGluPheLeuValGlnAlaTrpLysSerLysLysThrProMetGlyPheSerTyrAsp GTTGAATTCCCTCGTGCAAGCGTGGAAAGTCCAAGAAACCCCAATGGGGTTCTCGTATGAT CAACTTAAGGAGCACGTTCCGACCTTCAGGTTCTTTTGGGGTTACCCCAAGAGCATACTA

7921 ThrArgCysPheAspSerThrValThrGluSerAspIleArgThrGluGluAlaIleTyr
 ACCCGCTGCTTGACTCCACAGTCACTGAGAGCGACATCCGTACGGAGGAGCAATCTAC
 TGGCGGACGAAACTGAGGTGTCACTCTCGCTGTAGGCATGCCTCCTCCGTTAGATG

 7981 GlnCysCysAspLeuAspProGlnAlaArgValAlaIleLysSerLeuThrGluArgLeu
 CAATGTTGTGACCTCGACCCCAAGCCGCGTGGCCATCAAGTCCCTCACCGAGAGGCTT
 GTTACAACACTGGAGCTGGGGTTCCGGCGCACCGGTAGTTCAGGGAGTGGCTCTCCGAA

 8041 TyrValGlyGlyProLeuThrAsnSerArgGlyGluAsnCysGlyTyrArgArgCysArg
 (Gly)
 TATGTTGGGGCCCTCTTACCAATTCAAGGGGGAGAACTGCGGCTATCGCAGGTGCCGC
 ATACAACCCCGGAGAAATGTTAAGTTCCCCCTCTTGACGCCGATAGCGTCCACGGCG

 8101 AlaSerGlyValLeuThrThrSerCysGlyAsnThrLeuThrCysTyrIleLysAlaArg
 GCGAGCGCGTACTGACAACTAGCTGTGGTAACACCCCTCACTTGCTACATCAAGGCCCG
 CGCTCGCCGCATGACTGTGTATCGACACCATTTGTGGGAGTGAAACGATGTAGTTCGGGGCC

 8161 AlaAlaCysArgAlaAlaGlyLeuGlnAspCysThrMetLeuValCysGlyAspAspLeu
 GCAGCCTGTCGAGCCGAGGGCTCCAGGACTGCACCATGCTCGTGTGTGGCGACGACTTA
 CGTCGGACAGCTCGGCGTCCCGAGGTCCTGACGTGTACGAGCACACCCGCTGCTGAAT

 8221 ValValIleCysGluSerAlaGlyValGlnGluAspAlaAlaSerLeuArgAlaPheThr
 GTCGTTATCTGTGAAGCGGGGGTCCAGGAGGACGCGCGAGCCCTGAGAGCCTTCACG
 CAGCAATAGACACTTTCGCGCCCCCAGGTCCTCCTGCGCCGCTCGGACTCTCGGAAGTGC

FIG. 1V

FIG. 1W

8281	GluAlaMetThrArgTyrSerAlaProProGlyAspProProGlnProGluTyrAspLeu GAGCTATGACCAGGTACTCCGCCCCCTGGGGACCCCCACAAACAGAAATACGACTTG CTCCGATACTGGTCCATGAGCGGGGGGACCCCTGGGGGTGTGGTCTTATGCTGAAC
8341	GluLeuIleThrSerCysSerSerAsnValSerValAlaHisAspGlyAlaGlyLysArg GAGCTCATAAACATCATGCTCTCCAAACGTGTCACTCGCCACGACGGCGTGGAAAGAGG CTCGAGTATTGTAGTACGAGGAGGTTGCACAGTCAGCGGGTGTGCTGCCCGACCTTTCTCC
8401	ValTyrTyrLeuThrArgAspProThrThrProLeuAlaArgAlaAlaTrpGluThrAla GTCTACTACCTCACCCGTGACCCCTACAACCCCTCGCGAGAGCTGCGTGGGAGACAGCA CAGATGATGGAGTGGGCACTGGGATGTGGGGGAGCGCTCTCGACGCACCCCTCTGTCGT
8461	ArgHisThrProValAsnSerTrpLeuGlyAsnIleIleMetPheAlaProThrLeuTrp AGACACACTCCAGTCAATTCTCTGGCTAGGCAACATAATCATGTTTGGCCCCACACTGTGG TCTGTGTGAGGTCAGTTAAGGACCGATCCGTTGTATTAGTACAAACGGGGTGTGACACC
8521	AlaArgMetIleLeuMetThrHisPheSerValLeuIleAlaArgAspGlnLeuGlu GCGAGGATGATAGTACCCCATTTCTTTAGCGTCCCTTATAGCCAGGGACCGCTTGAA CGCTCCTACTATGACTACTGGGTAAAGAAATCGCAGGAATATCGTCCCTGGTCGAACTT
8581	GlnAlaLeuAspCysGluIleTyrGlyAlaCysTyrSerIleGluProLeuAspLeuPro CAGGCCCTCGATTGCGAGATCTACGGGGCTGTACTCTCATAGAACCACTTGATCTACCT GTCCGGGAGCTAACGCTCTAGATGCCCGGACGATGAGGTATCTTGGTGAACCTAGATGGA
8641	ProIleIleGlnArgLeuHisGlyLeuSerAlaPheSerLeuHisSerTyrSerProGly CCAATCATTCAAAGACTCCATGGCCCTCAGCGCATTTTCACTCCACAGTTACTCTCCAGGT GGTTAGTAAGTTTCTGAGGTACCGGAGTCCGGTAAAGTGAGGTGTCAATGAGAGGTCCA

8701 GluIleAsnArgValAlaAlaCysLeuArgLysLeuGlyValProProLeuArgAlaTrp
 GAAATTAATAGGTGCCCGCATGCCCTCAGAAACTTGGGTACCGCCCTTGCAGAGCTTGG
 CTTTAATTATCCCAACCGCGGTACGGAGTCTTTTGAACCCCATGGCGGGAACGCTCGAACC

 Gly
 8761 ArgHisArgAlaArgSerValArgAlaArgLeuLeuAlaArgGlyGlyArgAlaAlaIle
 AGACACCGGGCCCGAGCGTCCGGCTAGGCTTCTGGCCAGAGGAGGCGGTGCCATA
 TCTGTGGCCCGGCTCGCAGCGCGATCCGAAGACCGGTCTCTCCGTCCCGACGGTAT

 8821 CysGlyLysTyrLeupheAsnTrpAlaValArgThrLysLeuLysLeuThrProIleAla
 TGTGGCAAGTACCTCTTCAACTGGGAGTAAGAAACAAGCTCAAACTCACTCCAATAGCG
 ACACCGTTTCATGGAGAGTTGACCCGTCATTCTTGTTCGAGTTTGAGTGAGGTATCGC

 8881 AlaAlaGlyGlnLeuAspLeuSerGlyTrpPheThrAlaGlyTyrSerGlyGlyAspIle
 GCCGCTGGCCAGCTGGACTTGTCCGGCTGGTTCACGGCTGGCTACAGCGGGGAGACATT
 CGCGCACCGGTCGACCTGAACAGGCCGACCAAGTGCCGACCGATGTGCGCCCTCTGTAA

 8941 TyrHisSerValSerHisAlaArgProArgTrpIleTrpPheCysLeuLeuLeuAla
 TATCACAGCGTGTCTCATGCCCCGGCCCCGCTGGATCTGGTTTGTCTACTCCTGCTTGCT
 ATAGTGCACAGAGTACGGGGCGGGGACCTAGACCAAAACGGATGAGGACGAAACGA

FIG. 1X

FIG. 1Y

9001 AlaGlyValGlyIleTyrLeuLeuProAsnArgOP
GCAGGGGTAGGCATCTACCTCCTCCCAACCGATGAAGGTTGGGGTAAACACTCCGGCCT
CGTCCCCATCCGTAGATGGAGGAGGGTTGGCTACTTCCCAACCCCATTTGTGAGGCCGGA

() = Heterogeneity due possibly to 5' or 3'-
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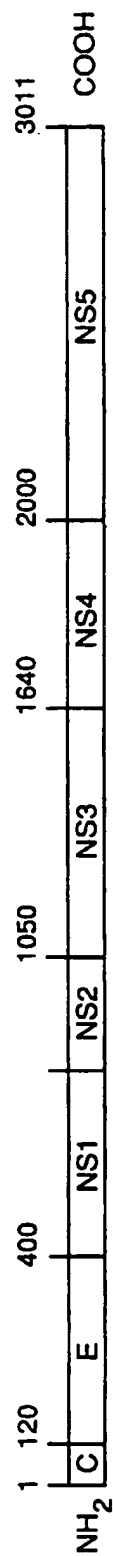


FIG. 2